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THE NEW PHYTOLOGIST

FOUNDED BY A. G. TANSLEY IN 1902

EDITED BY

A. R. CLAPHAM, H. GODWIN, W. O. JAMES

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THE NEW PHYTOLOGIST

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15 FEBRUARY, 1932

EDITORIAL

IN assuming the task carried out for thirty years by Prof. Tansley with such devotion and success, it is appropriate to redefine the undertaking as it appears to us. In the opening number of 1902 it was hoped that the journal might afford easy communication and discussion on all botanical subjects, publish stimulating suggestions arising as incidentals in research, notice important new literature, and keep the non-professional botanist and the worker, not attached to an active centre of research, acquainted with the latest developments.

Ten years later the scheme had translated itself into a stabilised practical form and the *New Phytologist* was publishing accounts of original research by British and, occasionally, foreign workers anxious to appeal directly to English-speaking botanists. In addition there were critical reviews of rapidly developing subjects, notices of recent books and discussions of educational and allied affairs. The journal had become a recognised channel for the publication of new contributions, but it had also established its claim to a catholic sympathy and a critical outlook over the whole field of botany.

It is in this sense that we conceive the duties we have accepted with the management of this journal. Two ends are to be served; contributions to botany both of facts and of ideas. It is inevitable that in the lapse of thirty years the best means of fulfilling these designs should have changed. The great advance of educational journals has made the appeal to teachers of less importance, but the production of critical reviews for the benefit of advanced students and research workers is as essential as ever. The discussion of new views as well as the reporting of new facts will therefore continue to be a main pre-occupation of the journal.

In conclusion it may be permitted to us to express our conviction of the magnitude of the service which Prof. Tansley has rendered to botany in founding the *New Phytologist* and in directing it through three decades.

NATURAL GRAFTING IN *HEDERA HELIX*

By M. ETHELWYN MILLNER, M.Sc.

(With Plate I and 13 figures in the text)

I. INTRODUCTION

THE phenomenon of natural grafting is a subject that has been practically untouched by botanists. Van Tieghem⁽⁵⁾ refers to natural grafting between branches and roots of neighbouring trees of the same species in many forests, and notes that if one of the branches be cut below the graft or the aerial parts be removed above a graft between two roots, the neighbouring tree nourishes the unrelated branch or root which lives attached to it. Dixon⁽³⁾ briefly mentions this question in quoting from Hales's work on the downward current of organic substances. Romell⁽¹⁾ mentions natural grafting between trees of the same species in Scots fir, maple, elm, beech, silver poplar and ash. He also describes an extraordinary natural graft between a young spruce branch and a Scots fir. Lastly, Caldwell⁽²⁾ describes an interesting natural graft between two forks of an elm tree. No reference has been found to natural grafting in ivy, which is, however, a very common phenomenon.

It is well known that when *Hedera helix*, growing on a tree or wall, is cut away here and there at the base, leaving part of the plant still intact, very little of the plant dies. In fact only those stems immediately adjacent to the cutting are seen to wither. On closer examination it will be noticed that here and there, where two or more stems are in contact with one another, fusion has taken place between them at the point where they touch. In old plants there may be a whole series of these grafts forming an anastomosing network of stems. It would seem probable that the upper branches are able to obtain water and nutrient salts from far distant stems, by means of this anastomosing network formed by the fusion of stems. This paper attempts to show how this takes place and to explain the structure of the grafts and the process whereby fusion has been brought about.

II. THE EXTERNAL CHARACTERS OF THE GRAFTS

Hedera helix varies somewhat in growth form according to habitat. It may be prostrate, or, in the presence of a suitable support, it may climb to a height of thirty feet or more. Stems, firmly attached to

a tree or wall, cross each other many times. Grafting occurs only in the climbing type where the stems are closely applied to some surface and where they are in contact with one another.

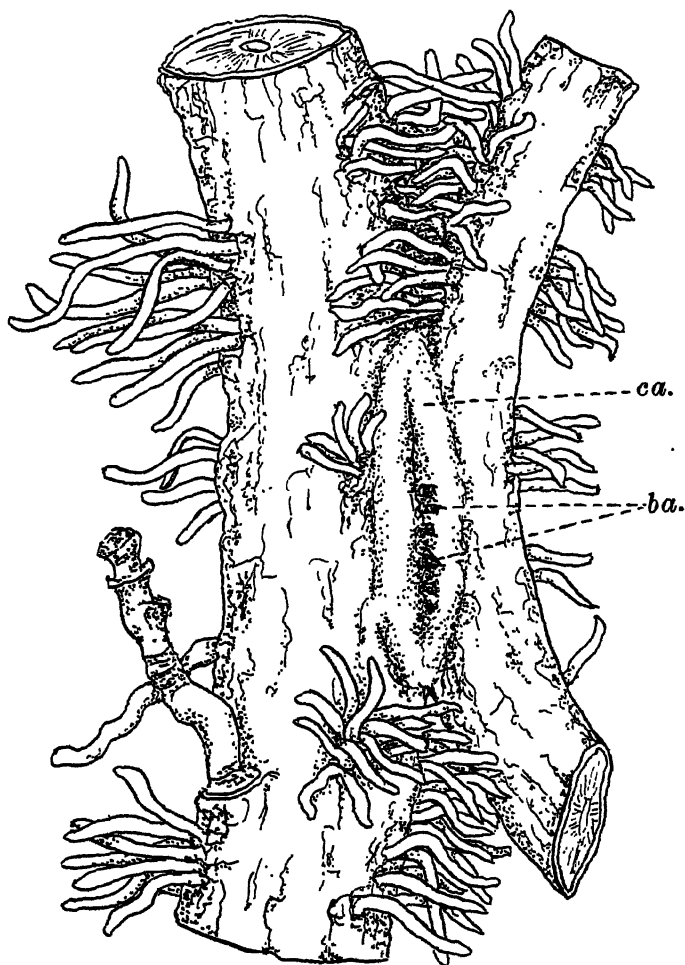


Fig. 1. Example of graft, for description see text. ($\times 3$.) *ba.* broken bark; *ca.* callus-like ridge.

Fusion may be found between stems that have grown side by side, or more frequently where one stem has crossed another. Grafts of all sizes occur in old ivy plants. At the base of the plant gnarled

stems two to three inches in diameter may be seen to have fused so completely that at the point of union they appear as one (Pl. I, phot. 1). There may be varying degrees of fusion according to the age and size



Fig. 2. Young graft, for description see text. ($\times 1\frac{1}{2}$.) *ca.* callus-like ridge.

of the stems, while often three or even more stems are found to have joined and formed a complete graft (Pl. I, photos. 2, 3 and 4). In young plants of four to five feet high, the stems are not so numerous and they appear to have grown fairly straight up the trunk of the supporting tree; consequently there is little, if any, grafting found. It



Phot. 1



Phot. 2



Phot. 3



Phot. 4

Phot. 1. Old graft where two stems appear as one except at a point in the middle of the stems where the original dividing groove can still be seen. Photos. 2, 3, 4. Examples of complex grafts between 3 or more stems.

MILLNER—NATURAL GRAFTING IN *HEDERA HELIX*

seems that firm anchorage of the stems to a surface, contact of the two stems, and then possibly subsequent compression is necessary in order to bring about grafting.

In old grafts the stems appear to be as one at the point of contact. This is seen in Pl. I, phot. 1, where the stems have completely fused.

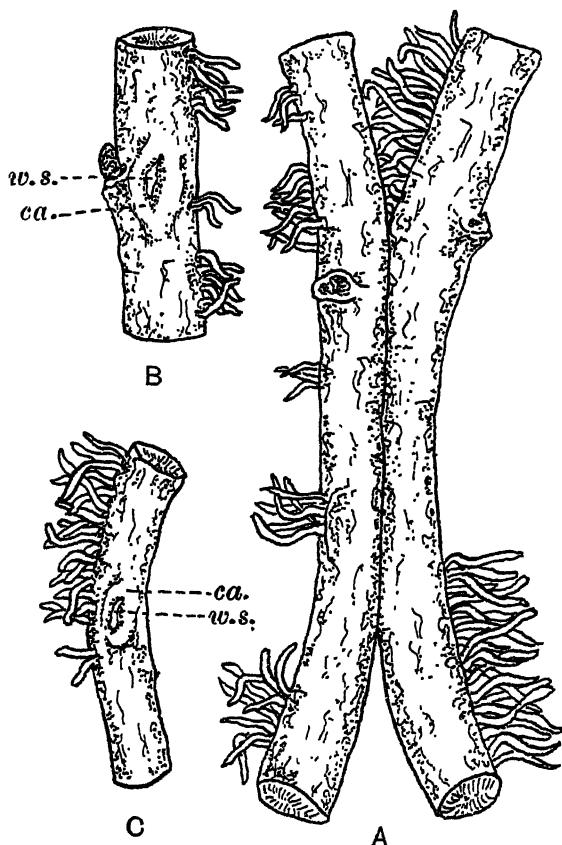


Fig. 3. Young grafts showing first stages of grafting. ($\times 2$.) *ca.* callus-like ridge; *w.s.* white strand.

In younger grafts, however, the groove between the two fusing stems is still visible (Fig. 1). In this groove there are often present small pieces of broken bark or epidermis (Fig. 1, *ba.*). This seems to indicate that the cork layers have been ruptured at some time, and are being pushed out as the fusion between the two stems becomes more complete.

On each side of the groove there are frequently present smooth ridges of tissue (Fig. 1, *ca.*). In stems which have climbed parallel to one another, there are therefore usually four such ridges, two on the exposed surfaces and two on the surfaces next to the support. But in grafts where one stem has crossed another, it is usually only the lower stem that forms these ridges, the upper stem appears to be pulled into the lower, by its adventitious roots growing firmly round it and it tends to flatten out over the lower stem (Fig. 2). In the very young stages, the stems are only connected by a fine white strand of tissue (Fig. 3, *w.s.*) and here the ridges are only just beginning to form. The formation of these ridges is probably due to the pressure one against the other which the actively growing stems produce as they increase in thickness.

The ages of individual stems of a grafting pair are rarely the same. However, they never seem to differ by more than five to six years, the difference averaging from two to three years. Early stages of grafting are always found in young stems of two to four years, and as the stems become older the grafts also mature. Grafting does not take place if a young branch grows across a very old one, neither does fusion occur if two stems eight or more years old come to lie close together. These old stems, probably owing to the thickness of their cork covering, are not injured by contact with each other, whereas, younger stems, by reason of their very thin cork layers become compressed by the contact and pressure of an adjacent stem. As these signs of compression in young stems appear to be the early stages of grafting, it seems that grafting only occurs in stems which have been in contact when young, before much cork is formed.

III. THE PROCESS OF FUSION BETWEEN TWO STEMS

The grafting of two *Hedera helix* stems is a complicated process. Sections across grafted portions show that the stems are united by a broad band of tissue, by which the cortex and vascular tissue of the one stem are connected with those of the adjacent stem.

On examination of young stages and also of compressed stems where no fusion has taken place, there appear to be two regions from which the connecting band arises: (*a*) the phellogen which gives rise to the cortical tissue and the cork, and (*b*) the cambium from which the connecting xylem and phloem are formed.

The initial stages of grafting are found in young stems which are in contact with one another and which show signs of compression

but no fusion. Where these stems touch the pressure of the one against the other causes a flattening or slight curve in their contours. The cortical cells appear crushed and very tightly pressed together, while the epidermis or a layer or two of the cork cells may become broken, perhaps by the rubbing of one stem against the other. At the point where the bark has been ruptured, the phellogen has cut off several layers of large cells with thin suberised walls (Fig. 4, *w.*). Beneath this tissue there may be seen a slight bulging of the phellogen and neighbouring cells. It is in this region that the first sign of the connecting band appears as a small protuberance.

The phloem also shows signs of compression, being narrower on the side of contact than elsewhere. Often the phloem and medullary rays may be slightly deflected away from one of the main medullary rays (Fig. 4, *e.m.*) which appears to widen as a result of pressure. This ray is usually situated opposite the point where the one stem is in contact with its fellow, and the angles of the deflections of the phloem and other medullary rays become less the farther they are removed from this point. The cambial cells across this wide medullary ray become more active here than elsewhere, and it is in this region that that part of the connecting band formed from the cambium arises.

The xylem does not become crushed like the softer tissues, but the cambium is stimulated to form small, thick-walled wood fibres, regular in size and arrangement, which stand out in marked contrast to the rest of the xylem (Fig. 4, *z.*). This tissue is widest in the region of the expanded medullary ray, narrowing off away from this zone till, in those parts where pressure is not felt, it is not formed.

Growth from the phellogen and cambium will be considered separately.

(a) *Growth from the phellogen*

The phellogen forms several layers of thin suberised cells at the point where the bark has been broken. The formation of this tissue gradually spreads from the point of wounding round the whole of the grafting side of the stem, becoming narrower away from this point. This tissue is of the nature of wound cork and differs from the normal periderm in the large size of its cells and the thinner walls which are only slightly suberised (Fig. 5, *w.*).

The phellogen in this grafting region is stimulated to form on its inner side, between it and the collenchyma, a phelloderm consisting of several layers of small thin-walled cells. On the flat side of the

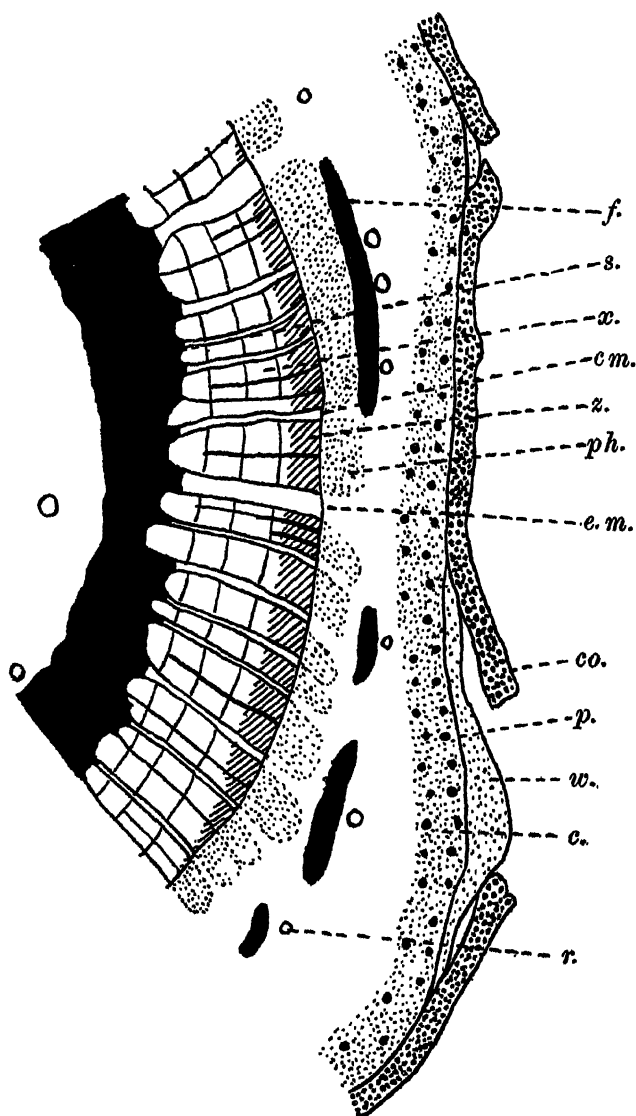


Fig. 4. T.S. of compressed stem. ($\times 65$.) *c.* collenchyma; *cm.* cambium; *co.* cork; *s.m.* expanded medullary ray; *f.* fibres; *p.* phellogen; *ph.* phloem; *r.* resin duct; *s.* sclerenchyma; *w.* wound cork; *x.* xylem; *z.* zone of thick-walled xylem.

stem this tissue consists of regularly arranged rows of cells. At the two edges of the grafting surface the cells become more numerous, larger and rounded, while the tissue loses its lamelliform formation, the cells becoming loosely and irregularly arranged (Fig. 5, *k.*). This

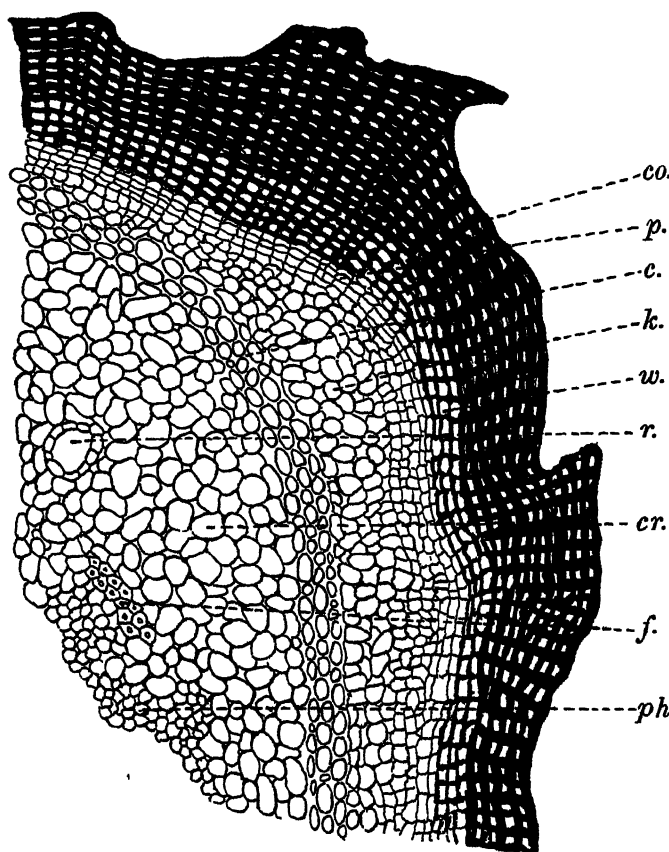


Fig. 5. T.S. of angular ridge found in grafting stems. ($\times 80.8$.) *c.* collenchyma; *co.* cork; *cr.* cortex; *f.* fibres; *k.* cells formed by division on the inside of the phellogen; *p.* phellogen; *ph*. phloem; *r.* resin duct; *w.* wound cork.

gives an angular appearance to the stem and eventually forms the ridges noticed on either side of the dividing groove between the two stems (Figs. 1 and 2, *ca.*). The collenchyma may thus become embedded between the original cortex and the phelloderm (Fig. 5, *c.*). Often, however, the ridges appear to be formed by the pressure from the adjacent stem causing the cortex and outer tissues to be pushed

outwards, away from the point of contact, where this tissue becomes very narrow, getting much wider at the edges of the stem where the ridges are thus formed. This may cause the collenchyma to become broken in various places.

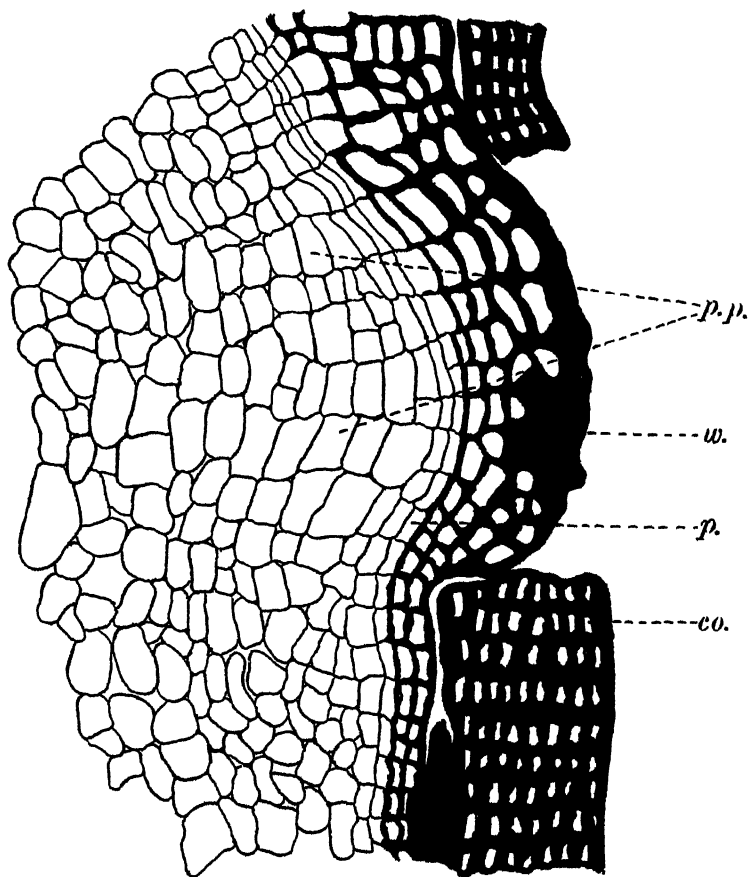


Fig. 6 TS of phellogen protuberance. ($\times 384$) *co.* cork, *p* phellogen; *p.p.* phellogen protuberance, *w* wound cork

At a point where the cork layers have become ruptured, which is often opposite or nearly opposite an expanded medullary ray, the thin-walled cells cut off on the inside of the phellogen, enlarge somewhat and consequently begin to bulge outwards. More thin-walled cells are cut off from the phellogen and a protuberance is formed (Fig. 6, *p.p.*) which grows by further divisions of the phellogen and

subsequent enlargement of the cells. It pushes through the already broken bark, taking the wound cork out with it and grows towards a corresponding protuberance which has in the meantime formed in the adjacent stem. Eventually, the protuberances burst through the wound cork.

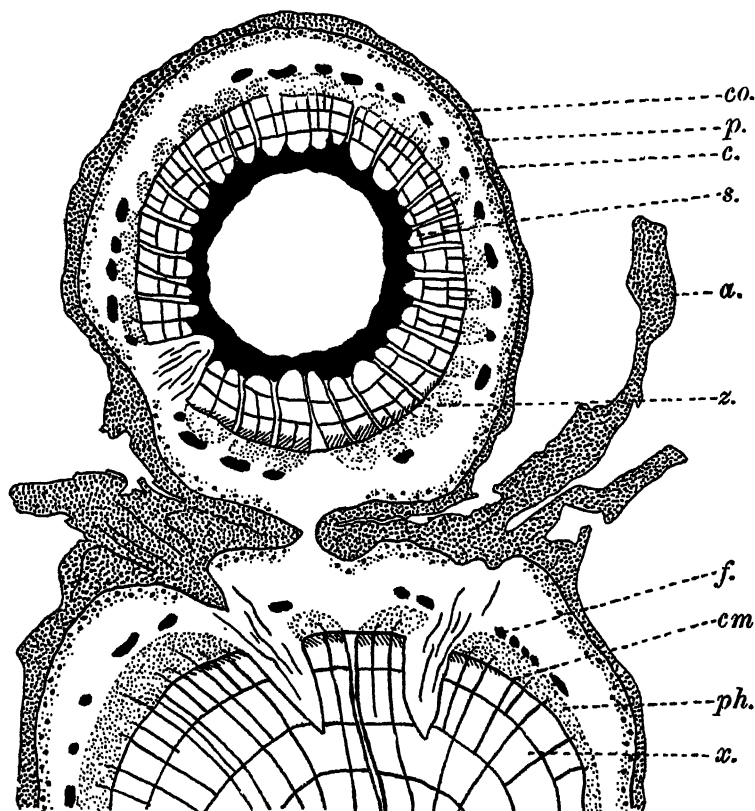


Fig. 7. T.S. of grafting stems where the phellogen protuberances have fused. ($\times 16.8$) *a.* adventitious root; *c.* collenchyma; *cm.* cambium; *co.* cork; *f.* fibres; *p.* phellogen; *ph.* phloem; *s.* sclerenchyma; *x.* xylem; *z.* zone of thick-walled xylem.

Almost as soon as the protuberances have broken through the wound cork covering them, the protuberance of one stem meets that of the adjacent stem and the two fuse, forming a narrow connecting band between the two stems (Fig. 7). This broadens by further division and growth from the phellogen, the newly formed cells enlarging and taking on the form of ordinary cortical cells. Thus they

push outwards the old periderm and the wound cork, fragments of which may be seen in the groove between the grafting stems (Fig. 1, *ba.*).

As the band widens the groove becomes obliterated, and eventually the grafting stems acquire a more or less even contour while the periderm becomes continuous round both stems owing to the old bark being shed. No new collenchyma has been observed to form in the connecting band. The old collenchyma has by now been broken through by the growth of a protuberance from the cambium, if not previously ruptured by pressure. Meanwhile changes have taken place in the region of the expanded medullary ray, and a protuberance has formed which grows outwards from the stele.

(b) *Growth from the cambium*

The first sign of growth from the cambium is seen in the expansion of a main medullary ray, as has already been mentioned. This seems to widen partly as a result of pressure from the adjacent stem and partly by the increase in size of its cells. The cambium at this point begins to divide more actively than elsewhere, forming a slight bulge (Fig. 8). The expansion of the medullary ray and the subsequent

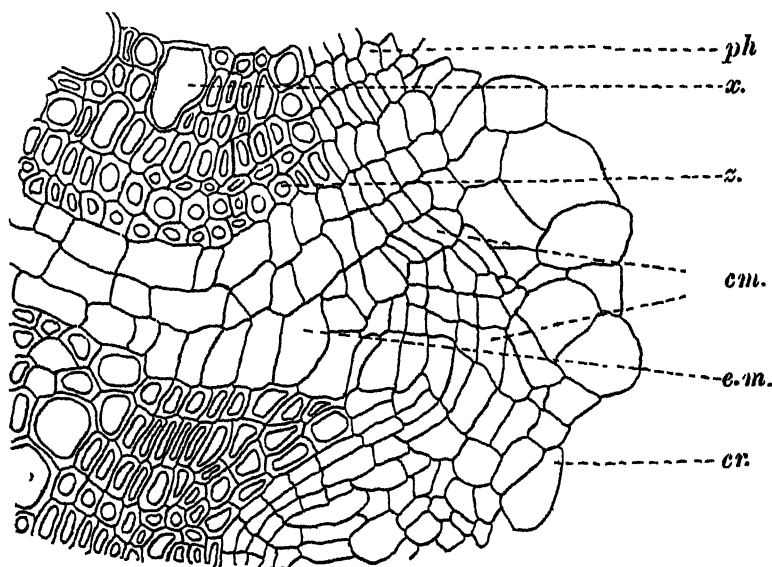


Fig. 8. Formation of cambial protuberance by division of the cells. ($\times 365.7$)
cm. cambium; *cr.* cortex; *e.m.* expanding medullary ray; *ph.* phloem;
x. xylem; *z.* zone of thick-walled xylem.

growth of the protuberance from it goes on simultaneously with the formation of the thick-walled xylem tissue mentioned above (Fig. 8). These small wood elements curve away from the medullary ray in

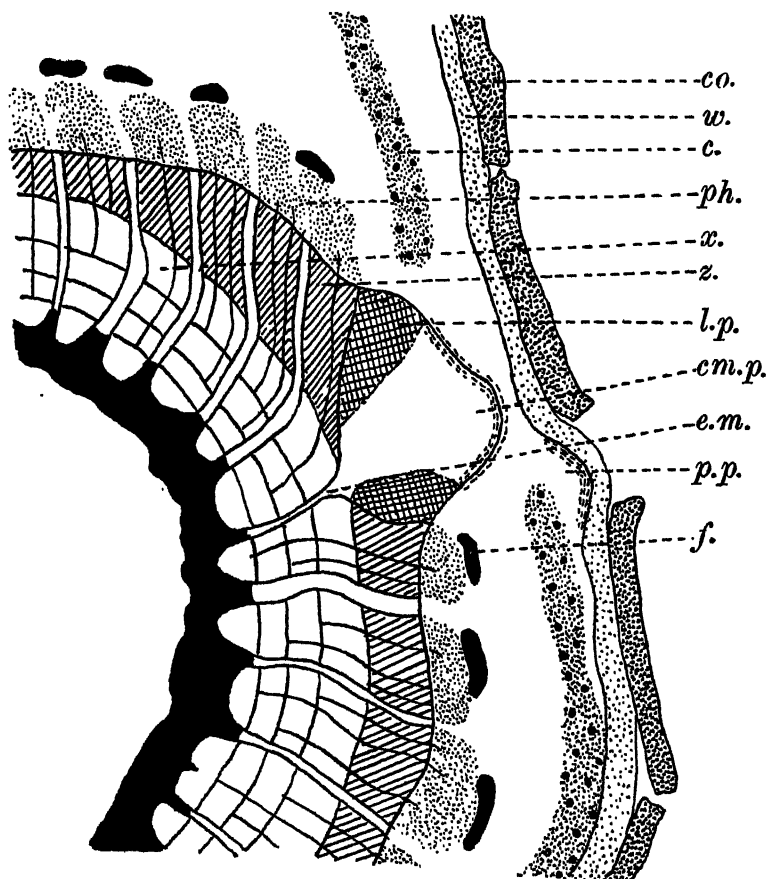


Fig. 9. Cambial protuberance showing beginning of lignification. ($\times 57.6$)
c. collenchyma; *cm.p.* cambial protuberance; *co.* cork; *e.m.* expanding medullary ray; *f.* fibres; *l.p.* lignified parenchyma; *ph.* phloem; *p.p.* phellogen protuberance; *s.* sclerenchyma; *w.* wound cork; *x.* xylem; *z.* zone of thick-walled xylem.

the same way that the phloem and other medullary rays bend away from this region (Fig. 9).

The cambium across this medullary ray eventually begins to bulge outwards, owing to the segmentation of cells tangentially on its inner side (Fig. 8). Division now also takes place radially, so that

the cambium is pushed outwards into the cortex forming a protuberance of cells (Fig. 9, *cm.p.*). This grows away from the centre of the stem, towards a corresponding protuberance formed in the adjacent stem. The protuberance consists of several layers of small narrow cells arranged in parallel rows, within which are large clear cells, loosely and irregularly arranged.

The protuberance continues to grow outwards by the growth of the cambium which now cuts off thin-walled cells on the outside as well as on the inside. It gradually pushes its way through the cortex and finally through the collenchyma (Fig. 9). The collenchyma remains as two tongues of tissue, at first clearly distinguishable in the cortex. However, the collenchymatous cells eventually begin to disorganise and appear as two darkly staining indistinguishable masses with no definite cell walls.

By the time the protuberance has reached the outer tissues of the stem, it will be noticed that the cells first cut off on the inside nearest the vascular cylinder show signs of lignification (Fig. 9, *l.p.*). The thickening is very gradual, the walls, though lignified, being at first not very much thicker than the adjacent unlignified cells. The cells which first show signs of lignification may have only part of their walls lignified, the walls that are towards the centre of the protuberance being unthickened, and often remaining so. Later-formed cells have all their walls lignified. The thickening gradually becomes more pronounced, and simple pits are seen in the walls. Eventually there are two groups, each of several layers of lignified parenchyma, formed on the inside of the protuberance (Fig. 9). The thickening of the cell walls spreads from the inside outwards as the protuberance grows (Fig. 10). The latter is by now much wider across, and the medullary ray opposite which it was formed will be seen to have expanded much more and the other medullary rays, phloem and cortical tissues will show signs of much greater compression. Likewise the zone of small lignified elements will have increased in width (Fig. 9).

The two protuberances from each stem will by now have grown through the cortex (Fig. 10) and across the connecting band which arose from the activity of the phellogen. Finally, the two meet and fuse with each other (Fig. 11). It seems that the cambium cells at the point where the two protuberances meet are simply converted into ordinary parenchymatous cells, and lose their meristematic activity, leaving a continuous ring of cambium common to both stems. Meanwhile the lignification of the cells first cut off has

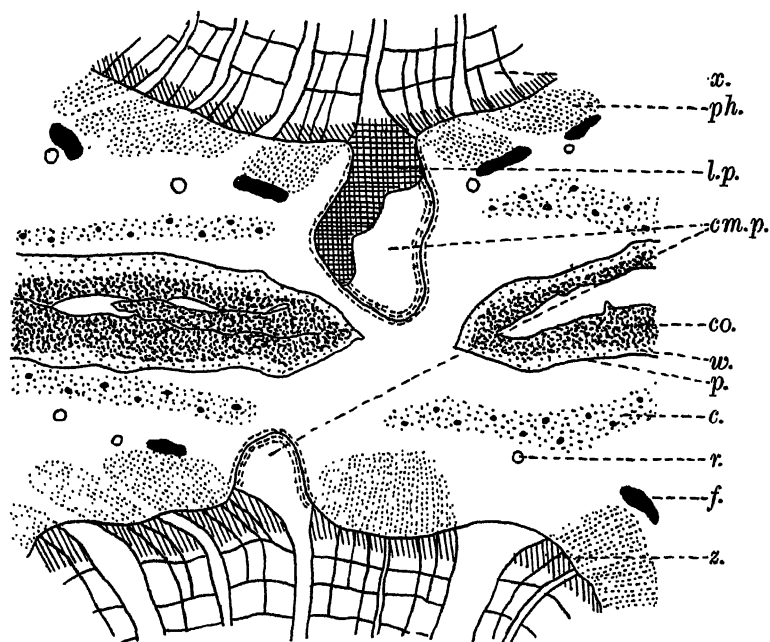


Fig. 10. T.S. of grafting stems showing growth of cambial protuberance ($\times 43.3$.) *c.* collenchyma; *cm.p.* cambial protuberances; *co.* cork; *f.* fibres; *l.p.* lignified parenchyma; *p.* phellogen; *ph.* phloem; *r.* resin duct; *w.* wound cork; *x.* xylem; *z.* zone of thick-walled xylem.

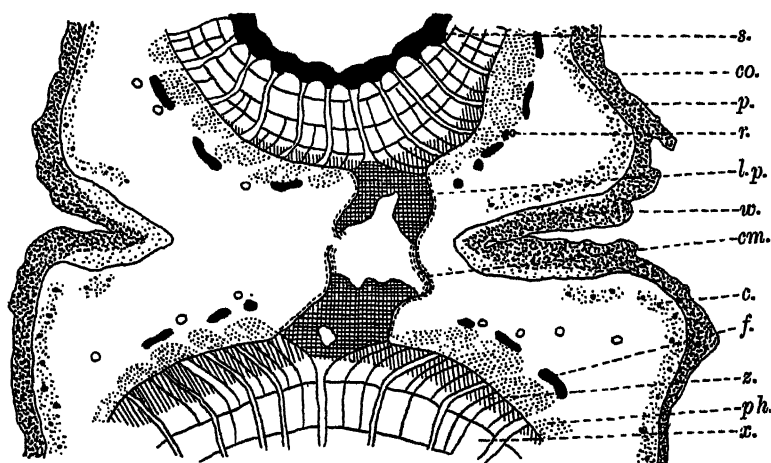


Fig. 11. T.S. of grafting stems showing the meeting of the cambial protuberance. ($\times 26.3$.) *c.* collenchyma; *cm.* cambium; *co.* cork; *f.* fibres; *l.p.* lignified parenchyma; *p.* phellogen; *ph.* phloem; *r.* resin duct; *s.* sclerenchyma; *w.* wound cork; *x.* xylem; *z.* zone of thick-walled xylem.

proceeded further, and finally nearly all the cells within the band formed by the cambium become thickened, except a mass of loose parenchymatous cells in the centre.

Differentiation of phloem and xylem elements may now be observed. The former appear first near the origin of the cambial protuberances and spread gradually across the band, so that the phloem of both stems eventually becomes continuous. The first formed

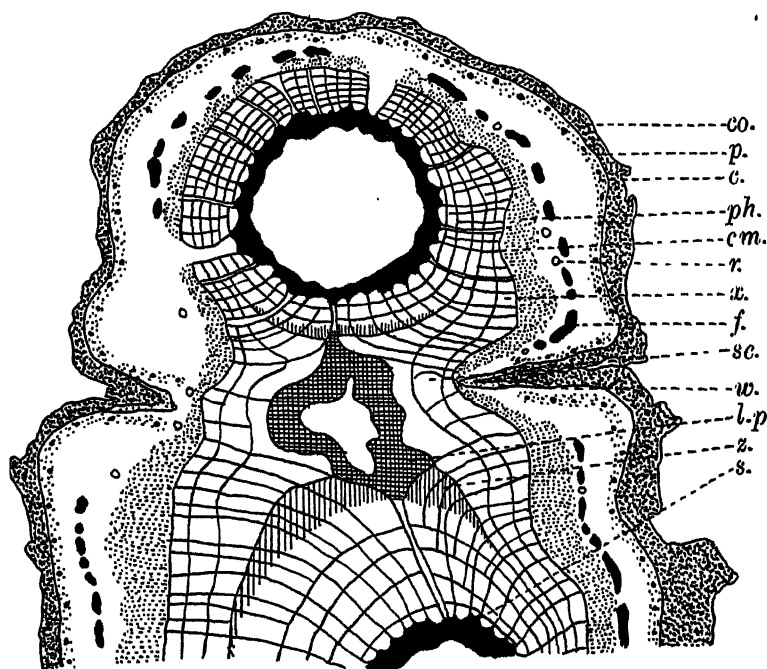


Fig. 12. T.S. of old graft. ($\times 14$.) *c.* collenchyma; *cm.* cambium; *co.* cork; *f.* fibres; *l.p.* lignified parenchyma; *p.* phellogen; *ph.* phloem; *r.* resin duct; *s.* sclerenchyma; *se.* common secondary wood; *w.* wound cork; *x.* xylem; *z.* zone of thick-walled xylem.

xylem elements consisting of tracheids and wood fibres are very small and distorted. As growth proceeds the tracheids become larger and more regular and straight. A complete band of xylem is eventually formed outside the lignified parenchyma, which encloses an irregular mass of unlignified cells (Fig. 12). Wood in the region of the graft is formed simultaneously with that in the stem so that common annual rings are formed round both stems (Fig. 12, *se.*). These are at first not so conspicuous in the connecting band, owing to the distortion of the cells, but eventually as the tracheids become

straighter and more regular, the rings are more pronounced. The annual rings in the region of the graft are at first broader than elsewhere, owing to the greater activity of the cambium at this point. This goes on until the groove between the two stems is obliterated, when the width of the annual rings becomes more or less equal round both stems.

The xylem elements across the graft are by no means regularly arranged, and the various component parts are very distorted as seen in longitudinal section. This may be due partly to the pressure exerted by the stems growing against each other, and partly to the fact that the stems rarely lie perfectly parallel to one another, so that the annual rings may be somewhat irregular as seen in cross-section. It is very common to cut through one stem exactly transversely, while the other stem and often part or all of the grafted portion is cut more or less obliquely.

The whole process of grafting is fairly rapid. It seems that the formation and union of the two pairs of protuberances and the formation of the lignified parenchyma in the connecting band thus formed, takes place during one season's growth. After this, growth goes on year by year in the same way that it does in the normal stem, so that the graft gradually increases in thickness.

There seems to be no definite order as to which protuberance is formed first, whether that from the upper or lower, or whether that from the larger or smaller stem. The phellogen protuberances are the first to appear and usually arise simultaneously. The cambial protuberances are not so regular and usually appear one before the other, but they generally form immediately after the phellogen protuberances. Occasionally they do not appear until after the fusion of the protuberances from the phellogens.

IV. OTHER FEATURES OF GRAFTS

Grafts, more complicated than those already described, may be found where three or four stems have fused together as in Fig. 13, which is a section taken from the grafted portion of the specimen seen in Pl. I, phot. 2. The pith centres of the three stems can be distinguished (Fig. 13, *a. b. c.*) and also the lignified parenchyma (*l.p.*), which was first formed between the grafting stems. Many such grafts may be found, in which the individual stems were of different ages when they fused, common annual rings being eventually formed round all the stems.

Grafting has frequently been observed in *Hedera helix* between

a stem and its branch when the angle between them was very acute. As both main stem and branch increase in thickness, this angle is gradually closed, so that the two are brought into close contact with one another, and grafting takes place between them.

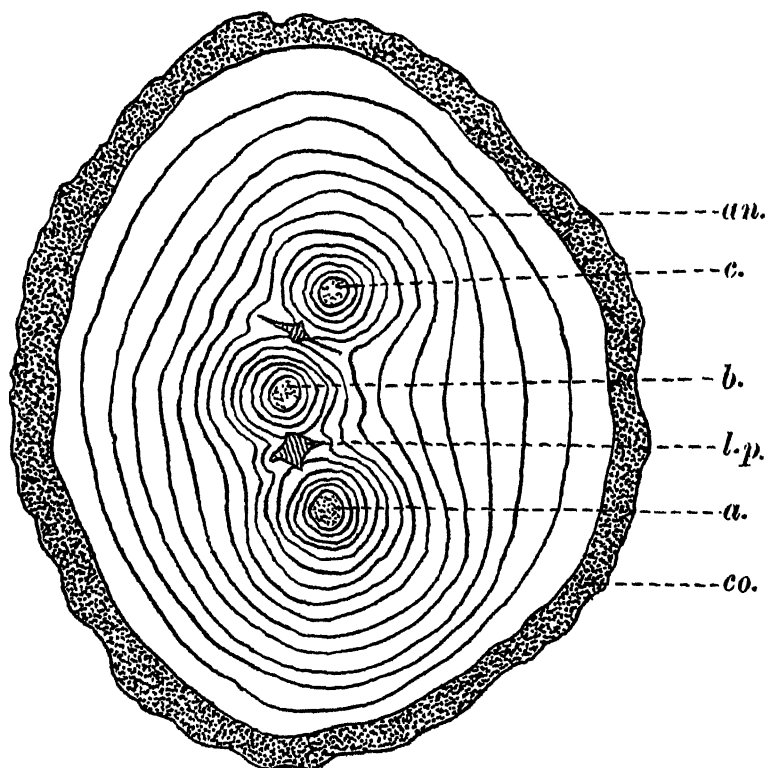


Fig. 13. T.S. of old graft between three stems, *a.*, *b.* and *c.* ($\times 3.5$) *an.* annual rings; *co.* cork; *l.p.* lignified parenchyma.

V. THE PASSAGE OF SAP ACROSS THE GRAFT

It was mentioned in the introduction that when part of a *Hedera helix* plant is cut away very little dies, and it was thought that this might be due to the conduction of sap across the grafts from one part of the plant to another. Experiments were therefore performed to ascertain whether the sap is able to cross the grafts, and if so how exactly it is transferred from one stem to the other. A dilute aqueous solution of eosin was used to trace the course of the ascending current.

One member, in each of several pairs of grafting stems growing naturally, was cut under eosin solution two or three inches below the graft, placed in a test-tube of this solution and left for several hours, after which each of the remaining stems was cut a few inches above the point of union. The age of the individual stems, their diameter and the angle they made with one another were observed. The grafts were then examined for the amount of eosin in each stem, by peeling off the outer tissues of the stem and the grafted portions, leaving the wood exposed, and by cutting sections at various levels with a small fretsaw.

It was found that the current passed up the stem which was placed in eosin solution and crossed the grafted portion into the adjacent stem. It was always confined chiefly to the side of the stem where the graft was present and passed upwards and downwards on that side of the stem. If the experiments were left for a short time, only the outermost elements nearest the graft of the second stem were coloured, but if left for a longer period the eosin appeared to diffuse from this through the outer elements of the stem.

On examination of the grafted portion, it was found that the eosin, in passing from one stem to another, was confined to the outer parts only of the connecting band where annual rings had been formed common to both stems, the lignified parenchyma in the centre of the band remaining colourless. Further evidence that the sap traverses the grafts by means of the common secondary wood, was seen on examining young grafts in which no common annual rings had formed. In these no eosin passed from one stem to the other. This is very much the same as described by Daniel⁽²⁾ in artificial grafts where the first-formed elements do not take part in conduction. The eosin seems to penetrate first through the large tracheids (there being no vessels) that are formed in the spring wood, as these are the first to become coloured. Later it diffuses through these into the smaller elements. This accounts for the stain often being present in alternate faint and dark bands, the latter marking the position of the larger elements, the former of the smaller elements.

The amount of eosin that crosses the graft seems to depend partly on the number of large tracheids in the grafted portion, more eosin crossing to the adjacent stem when there was a large amount of common wood and consequently a greater number of large tracheids than when there was a smaller amount of wood in the grafted region and therefore fewer tracheids. This of course depends on the age of the grafts. Experiments were performed in which young and old

grafts were left with one stem in eosin solution for an equal period of time. More eosin passed across the older grafts with the larger number of tracheids, and consequently more was found in the adjacent stem when the grafted portion between the two was fairly old.

The actual age of the grafted portion, rather than the age of the individual stems, seems to affect the amount of eosin passing across the graft. In experiments where either the younger or the older member of a pair was placed in eosin solution, the age of the individual stems appeared to have no effect on the amount of eosin passing across the graft. This depended always on how far grafting had advanced in the pair selected.

The angle that the two grafting stems make with one another seems also to influence the amount of eosin crossing the graft. Daniel⁽²⁾ has shown that a branch at an acute angle will receive more liquid in a given time than a similar branch which is at a less acute angle. This is seen also in natural grafting. When two fused stems are practically parallel, or the angle between them is very acute, the amount of eosin in the adjacent stem is far greater than when the stems join at right angles to one another, when the amount of eosin passing into the second stem is very small.

The amount of eosin that passes downwards in the stem into which it has diffused, is usually less than that which passes upwards. If, however, one or both of the upper stems is injured in any way or dies, it seems that the amount of sap going down the stem is increased. Experiments were set up in which either or both of the upper stems were removed, the cut ends being sealed up with wax, and it was noticed that the downward movement of the eosin was increased.

The amount of eosin passing from one stem to another appears also to be influenced by the presence of leaves on the stem above the graft, and the consequent pull on the transpiration stream in these stems. Leaves were stripped off one stem above a graft, the other of the pair being placed in eosin. A similar experiment was set up as a control, no leaves being removed. In a given time the amount of stain which diffused into the leafless branch was less than that which had passed across into the leafy branch of the control graft. It seems that when there are only a few leaves on the stem above the graft, the pull on the transpiration stream being small, less eosin is drawn across the graft than into a more leafy stem in which the pull from above is greater.

In order to ascertain what part of the ascending stream in the stem passes across the connecting band into the other stem, different

portions of the base of several stems were removed for an inch. The cut surfaces thus made were sealed with wax, but the bottom of the remaining portion was left unscaled and placed in eosin. In this way the ascending sap was confined to different regions of the stem in different experiments. It was found that in normal stems that part of the ascending current nearest the adjacent stem passes across the connecting band and into this stem, but if the current at this point is stopped artificially the ascending sap from other parts of the stem can slowly diffuse outwards radially and so across the grafted portion.

It has thus been shown that the sap can pass from one stem to another across the graft and then upwards and downwards in the second stem. Where there is a whole network of such grafts on a tree, this passage of sap from one stem to another would account for the fact that when stems of such a *Hedera helix* plant are cut away in one or two places, only the branches adjoining the cuts wither, the others are able to get their necessary water from other parts of the plant.

VI. THE CAUSES OF FUSION BETWEEN TWO STEMS

In order to throw light upon the causes of fusion several attempts were made to graft stems artificially. Pairs of *Hedera helix* stems were selected which had not previously been touched. Slight incisions in the bark were made on the adjacent sides of pairs of stems and the wounded surfaces were placed in contact, both stems being bound firmly together. Other pairs of stems were treated in a similar way, but the wounds penetrated into the inner layers of the cortex, or even as far as the vascular system. Other pairs of stems were taken and bound together without wounding.

It was found that the stems that had been wounded deeply had reacted to the stimulus of wounding in the normal way, each wound healing separately. Grafting was gradually proceeding, however, between pairs of stems whose cork layers had been broken at the point of contact. At each point a wound cork, and below this a conspicuous protuberance had formed, whilst there was sometimes evidence of growth from the cambium. These observations resembled exactly the phenomena found in stems grafting naturally.

One or two of the pairs of stems which had not been wounded showed the first stages of grafting, though others showed no signs of change in structure. These changes were only seen on examining the internal structure, when it was found that there was a break in

the bark at the point of contact of both stems, and at these points protuberances had formed. Cambial protuberances had also formed in some of the stems. Once or twice stems were found with the bark broken at a certain point and forming only a very narrow gap. Here, although no actual protuberance had formed as yet, there was a slight bulging of the cells at this point, which seemed to indicate that the rupturing of the bark occurred before the formation of the phellogen protuberance, the latter arising as a result of this break in the periderm.

It thus seems clear that rupturing of the bark in some way definitely occurs in the natural grafting of two stems, and since this appears to take place before the formation of a phellogen protuberance it seems probable that this must be due to some external influence and not to the protuberance forcing its way through the bark. It would therefore appear from the results of all these experiments that slight wounding is the stimulus which initiates grafting, in the same way that Daniel(2) describes artificial grafting and Van Tieghem(5) grafts between tree branches as due to wounding and contact.

It must now be considered how the cork layers become ruptured at the point of contact of two stems. In forest trees wounding occurs by the friction of one branch against another, but the possibility of two adjacent stems of *Hedera helix* rubbing against one another is more remote as the stems so soon become fixed by means of their adventitious roots and also grafting only occurs in stems that are closely applied to one another and fixed to some surface.

However, there would be a slight friction as a young branch was growing across an older one until the former became anchored by its roots, which is not until the stems are two years old or more; but whether this friction would be sufficient to rupture the cork layers would depend upon their thickness and consequently on the age of the stems. Grafts between old and very young stems are not found, also experiments on the age of grafting stems show that an old stem with thick bark and a very young one are unable to graft together unless previously wounded at the point of contact. It therefore seemed improbable that the friction from a young branch could cause the rupturing of the thick periderm of an old stem, and that grafting only occurred if the lower stems were fairly young.

Cases have been found, however, where grafting did not begin in two stems until both were three or four years old, when the stems would be firmly fixed, so that wounding could not take place as

described above. Contact of two stems seems to inhibit to some extent the increase in thickness of the periderm, so that twigs lying near together and brought into contact by the increase in thickness of their stems may still have thin bark at the point where they touch. Secondary thickening of the twigs, firmly held together by roots, would produce an increase in pressure, and unequal root anchorage combined with this might cause a sliding of one over another, thus bringing about a slow grinding action between the two stems, which might damage the cork.

It seems that this wounding of the bark causes a release of pressure which stimulates the phellogen and cambium to grow outwards and form protuberances, in the same way that a wound callus forms at a cut surface as a result of the cambium being relieved from the compressing influence of the bark, as described by Ward (6). The latter also shows that the formation of the uniting callus of artificial grafts is due to the release of the pressure of the cortex, causing the cambium to grow out forming a callus which unites the two plants. Thus we have a further analogy between natural and artificial grafting and both seem connected with the problem of wound healing, since the callus in each case forms as a result of the release of pressure.

VII. CONCLUSION

There is a striking resemblance between natural and artificial grafting, especially in "grafts by contact" as they may be called. In the former a small wound occurs probably by the rubbing of one stem against another. In the latter cuts or incisions are made and the wounds thus formed are placed together. In both, the stimulus of wounding brings about increased activity in the phellogen and cambium. By active growth from the phellogen and cambium a union is made between both stems. At first this is only slight, but as growth proceeds the connection becomes more complete and there is a definite transference of sap from one stem to the other in both natural and artificial grafting.

The growth from the phellogen and cambium may be compared to a healing callus. Instead of each wound that is made healing separately in the normal way, the two healing calli unite with one another, so that there is a healing in common of two wounds. In fact grafting may be said to be a healing in common of two wounds.

VIII. SUMMARY

1. Grafting is found in stems which, when firmly attached to a support, lie in contact with one another.

2. It appears to start when the stems are fairly young, and old mature grafts, where the stems appear as one, are found in old thick stems.

3. Very young stems lying in contact, but where no fusion has as yet taken place, show marked compression and also structural change, such as the deflections of the phloem and medullary rays away from an expanded medullary ray, and the formation of a zone of small, thick-walled xylem elements. These appear to be the initials of grafting.

4. The bark becomes ruptured at the point of contact and the phellogen is stimulated to form extra tissue and also a protuberance which, by the enlargement of its cells and further division, grows towards a corresponding one formed in the adjacent stem and fuses with it. The band thus formed gradually widens.

5. Meanwhile the cambium across the expanded medullary ray bulges outwards and by continuous division forms a protuberance of cells, which grows outwards and across the connecting band formed by the growth of the phellogen. The cambial protuberance cuts off cells on the outside and on the inside, the latter gradually becoming lignified. Eventually this meets a corresponding protuberance in the adjacent stem and fuses with it, and finally a complete band of lignified parenchyma is formed.

6. Finally tracheids and phloem are formed. The former are very irregular at first, but gradually become straighter, and secondary thickening takes place so that annual rings are formed common to both stems.

7. Grafting in the angle between a stem and its branch, and the structure of grafts where two or more stems have fused, are described.

8. The conduction of liquids across the graft is considered, and it is shown that sap can pass from one stem to another by means of the large tracheids in the secondary wood formed common to both stems, and that the amount of sap passing from one stem to another is affected by various conditions.

9. The causes of fusion and various experiments to elucidate them are discussed, and it is shown that the wounding of the bark occurs, probably by the rubbing of the stems against one another.

10. This account of natural grafting in *Hedera helix* is concluded

with a comparison of natural grafting with artificial grafting, and it is shown that grafting is a healing in common of two wounds.

Before concluding, I should like to add that throughout the course of this research, I have been indebted to the late Prof. R. H. Yapp for suggesting this investigation and for his valuable criticism and help in every way. I also wish to thank Dr W. Leach, and Dr W. B. Turrill who kindly assisted me in preparing this paper for publication.

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THE INFLUENCE OF TEMPERATURE ON THE RATE OF ACCUMULATION OF CHLOROPHYLL IN ETIOLATED SEEDLINGS

BY V. N. LUBIMENKO AND E. R. HUBBENET

(With Plate II and 5 figures in the text)

THE influence of temperature on the greening processes of etiolated seedlings of Angiosperms has not been experimentally studied since the time of Wiesner, when he derived his conclusions as to the rate of accumulation of chlorophyll from direct observations on the colour of the seedlings.

The reason for this lack of investigation is the difficulty in the quantitative determination of the pigment; especially as the study of the greening process demands, in the early steps of its formation, manipulations with minimal quantities of chlorophyll, for the determination of which neither the method proposed by Willstätter nor certain macro-methods adopted in biochemistry can be used.

The colorimetric method, which would seem the best to employ is also impossible to use in this case, because the etiolated seedlings contain a large quantity of yellow pigments; and in extracts of chlorophyll obtained by the usual solvents, these pass into the solution and mask the coloration due to chlorophyll in the first steps of greening.

The only practicable method in this case is the spectrophotometric one, based on the observation of the main absorption band of chlorophyll. By this method it becomes possible to use the ordinary alcohol extracts of seedlings and to determine minimal quantities of chlorophyll, even though yellow pigments are mixed with it, as these have no bands of absorption in the red part of the spectrum.

Employing an extract of crystallisable ethyl-chlorophyllide as a standard solution, we can, by adopting the spectrophotometric method, express the accumulation of chlorophyll in percentages of its weight.

E. R. Hubbenet has used this method in her first paper on the influence of temperature on the greening of etiolated seedlings. Her experiments on seedlings of wheat and barley seem to show that in etiolated seedlings exposed to light the rate of accumulation of

chlorophyll must be controlled, not by the photochemical processes, but by catalytical reactions independent of light.

The results of this first investigation having been published in the form of a preliminary communication, the work has been continued on a somewhat different plan.

As the spectrophotometrical method allows of the determination of extremely small quantities of the pigment, the detailed study has been undertaken of the influence of temperature from minimum to maximum, on the rate of accumulation of chlorophyll in etiolated seedlings.

Wiesner has shown that definite minimum and maximum temperature limits exist for the greening of etiolated seedlings; but his definition of these limits could not be exact, as the absence of a clear green tint in seedlings cannot serve as a sufficient criterion for the total absence of chlorophyll. The exact definition of the limits can be obtained in this case only by an exact determination of the quantity of chlorophyll present independently of the yellow pigments. That is why we have undertaken in our new experiments the exact definition of the temperature limits for the processes of chlorophyll accumulation.

Following that, it was interesting to find the temperature coefficients of the rate of this process at different temperatures, and for varying lengths of time of exposure of the seedlings to light. The definition of the temperature coefficients presented a special interest for the explanation of the mechanism of the greening process.

In germinating seedlings chlorophyll is certainly formed from an uncoloured substance, the hypothetical leucophyll.

In plants of lower organisation, from Algae to Gymnosperms, the transformation of leucophyll into chlorophyll occurs in the absence of light. In the Angiosperms, on the contrary, this transformation absolutely demands the presence of light. The opinion of Liro and Tsatchenko is that the transformation of leucophyll into chlorophyll occurs in these latter plants quite photochemically and can be brought about even in the tissue killed by freezing or drying.

The formation of chlorophyll in etiolated and carefully dried seedlings, if they are exposed to light, has been confirmed by the studies of Monteverde and Lubimenko, but they have shown that it was due not to the transformation of leucophyll, but of a green pigment, standing optically near to chlorophyll. This pigment, discovered by Monteverde in etiolated seedlings, was first named protochlorophyll and afterwards chlorophyllogen.

Chlorophyllogen is present in etiolated seedlings in very small quantity compared with the yellow pigments, and cannot, for that reason, affect the yellow colour of the seedlings. If they are exposed to light their chlorophyllogen is immediately transformed into chlorophyll. This transformation of chlorophyllogen presents precisely the same photochemical process as is accomplished in the tissue of the dried seedlings. No new formation of chlorophyll by the transformation of leucophyll takes place, and that is why the seedlings do not change their original yellow colour. The change of colour under the influence of the new formation of chlorophyll can be observed only in living seedlings, if their exposure to light is prolonged for 1 hour or more.

Concerning the interior mechanism of the greening in Angiosperms, the fact that the accumulation of chlorophyllogen in the dark ceases as soon as it attains some definite small quantity is important. If the etiolated seedlings are for a short time exposed to light, and all their store of chlorophyllogen transformed into chlorophyll, after which they are again put into darkness, they accumulate the same limit-quantity of chlorophyllogen, but this process is a slow one, the limit quantity of pigment being obtained after 5-8 hours. Such an experiment can be repeated several times, always with the same result.

These facts allow the greening process to be represented by the following sequence of changes:

- (a) Synthesis of leucophyll.
- (b) Transformation of leucophyll into chlorophyllogen.
- (c) Transformation of chlorophyllogen into chlorophyll.

The first two reactions occur in the dark, but their course is stopped as soon as the quantity of chlorophyllogen attains some limit quantity. The last reaction is purely photochemical.

The study of the influence of temperature on greening is the study of the influence of this factor on the first two reactions, those proceeding in the dark, the photochemical processes being scarcely influenced by temperature.

The technique of our experiments was as follows: Carefully selected seedlings of a pure line of wheat, *Triticum ferrugineum* (N. 10879¹), were first moistened for 36-48 hours between wet leaves of filter paper and then planted in flat wooden boxes filled with soil;

¹ The seed material has been received from the stores of the Institute of Applied Botany, with the help of K. A. Flauburger, whom we are pleased to thank heartily.

these were 14 cm. long, 3.5 cm. broad, and 10 cm. high. In each box 12-15 seeds were placed, so as to obtain one line of seedlings in the box. The soil was first made very damp and its dampness was maintained at 80 per cent. of complete humidity.

Seeds were sown in 20 such boxes which were all placed in one big wooden box, covered with black material and taken into a dark room. The germination continued for 8-10 days, counting from the moment of moistening the seeds, at a temperature of 17-19° C. Seedlings 8-9 days old, grown to a length of 14-18 cm., were found to be most suitable for exposure to light. The seedlings of this age were moved, in their boxes, into a room kept at the desired temperature, and were illuminated by an electric lamp of 32 candle-power.

In order to obtain the same intensity of illumination for all the boxes, these were placed on a table in a circle of 1 m. diameter. The lamp was placed on a block in the middle of the circle, at a height of 25 cm. from the table, so that the plants were 55 cm. from the source of light. The table was covered with white paper, and the circle of boxes containing the plants was surrounded by a high wall of white cardboard, in order to obtain the same conditions of reflection for all the plants. Experiments were performed simultaneously with two groups of seedlings in two dark rooms, the difference in temperature of these being 10-11° C.

The time of the illumination of the seedlings varied from 2-72 hours, the quantity of chlorophyll being determined after 2, 8, 16, 24, 48 and 72 hours of exposure to light. The plants were transferred an hour before the beginning of illumination, in order that they should come to the temperature of the room.

The comparatively feeble intensity of illumination had been purposely chosen to avoid the further effect of light in destroying the chlorophyll which was formed. This destructive action of light, as has been shown by the experiments of Lubimenko on etiolated seedlings, begins with comparatively feeble intensities and increases rapidly with the brightness of illumination; that is why we have preferred to work with a feeble light.

As the transformation of chlorophyllogen into chlorophyll occurs immediately, even under feeble illumination, it was necessary to take the utmost care that the seedlings should never be exposed to light during their growth and removal to the experimental chambers. In this respect the spectroscopic test is very useful. Ten seedlings are removed in the dark, and are pounded with alcohol in a mortar, this

operation being carried out in darkness also, an alcoholic extract of the pigments being thus obtained. With the aid of the spectroscope we can readily find traces of chlorophyll in such an extract by the presence of its first absorption band. The total absence of the band and the presence of the first protochlorophyll band ($\lambda 640\mu\mu$ – $\lambda 620\mu\mu$), derived from chlorophylligen in the alcoholic extract, shows that the seedlings have not been affected by light prior to the experiment.

As macerating in darkness is not very easy, prolonged soaking in alcohol provides an alternative, or, using a simpler method, the seedlings can be killed in the dark by plunging them into boiling water, and can then be brought into the light, for the extraction of their pigments, the water having been first removed by pressing the seedlings between leaves of filter paper. To facilitate the operations in the dark one can also, as experience shows, use a feeble green light, which is least active in the transformation of the chlorophylligen.

Experiments by Greilakh and Lubimenko have shown the existence of a minimum light intensity for the transformation of chlorophylligen into chlorophyll; if the illumination is less than this minimum, the reaction does not occur. But before using green light for the work in the dark rooms it is necessary to establish, by preliminary experiments, the minimum intensity for green light, and to use illumination less than this. After exposure to light, as described above, a sample of seedlings was removed for the quantitative analysis of chlorophyll; for this purpose, 8–10 seedlings were cut off at two-thirds of their height, and 5 grm. were weighed. Then the seedlings were killed in boiling water, and their pigments extracted by pounding in a mortar with 90 per cent. alcohol. The volume of the extract was brought to 20 c.c. and the quantity of chlorophyll in it determined by comparison with the standard solution of ethylchlorophyllide, using a spectrocoulometer of new construction (p. 47).

The most difficult operation in this experiment is the accurate weighing of the living seedlings, which must be done under a much feebler light than that which illuminated the seedlings during the experiment. We made the weighing correct to the third decimal place. Each determination of the chlorophyll content was repeated for another sample of seedlings that had been similarly treated, in order to obtain a more exact result.

RESULTS OF THE EXPERIMENTS

We will give first the results of experiments which show that the greening of wheat seedlings does not occur at a temperature of 2° C.

The following table gives the quantities of chlorophyll found in two samples of seedlings, of which one was illuminated at 2° C., and the other at 11° C.

Duration of exposure of seedlings to light. Hours	Quantity of chlorophyll in mg. per kg. of fresh wt. of leaves	
	2° C.	11° C.
2	16.3	26.4
8	14.4	88.5
16	12.8	196.0
24	13.4	311.0
48	14.3	582.0
72	13.3	923.0

These figures show clearly that, at a temperature of 2° C. and lower, no new formation of chlorophyll takes place; the primary store of chlorophyllogen, accumulated in seedlings during their growth in the dark, is transformed into chlorophyll, and the small quantity thus produced remains unchanged during 3 days of illumination, or may even diminish, whilst at 11° C. the seedlings accumulate during that time nearly 1 gram. of chlorophyll for every kilogram of their fresh weight.

It was interesting to establish whether seedlings, when exposed to light for a long time at a low temperature, retain their capacity for forming chlorophyll.

One sample of seedlings was taken into a room kept at 11° C., after having been kept for 48 hours at 2° C., and after 24 hours they had accumulated 0.343 gram. of chlorophyll per kg. of fresh weight, an even greater quantity than the control one (0.311 gram.), placed directly in a room at 11° C. So undoubtedly the influence of temperature in the process of greening is the influence of this factor, not on the photochemical transformation of chlorophyllogen into chlorophyll, but of the reactions taking place in the dark, which precede the formation of chlorophyllogen.

We need not describe in detail the following numerous experiments, but will give in a table the quantities of chlorophyll obtained at different temperatures and after different periods of illumination, in mg. per kg. of fresh weight of leaves.

The figures given below are expressed as simple fractions; the numerators show the quantities of chlorophyll present at the end of

the experiments minus the quantities obtained by the transformation of the original store of chlorophyllogen; the denominators show the increase of chlorophyll during one hour's exposure to light.

Temp. ° C.	Duration of exposure of seedlings to light					
	2 hr.	8 hr.	16 hr.	24 hr.	48 hr.	72 hr.
4	0.7	2.8	—	37.6	129.5	218.0
	0.35	0.35	—	1.56	2.70	3.00
10	8.8	67.0	154	215	872	1050
	4.4	7.0	9.6	9.0	18.1	14.5
16	32.7	257	581	835	1503	1951
	16.4	32.0	35.6	34.8	31.3	25.6
20	41.3	445	1005	1064	1452	—
	20.6	55.6	62.8	44.3	30.0	—
26	137	756	1122	1323	2137	2362
	68.4	94.5	70.1	55.0	44.5	32.8
30	109	818	1072	1199	1295	—
	54.3	102.1	66.9	49.9	27.0	—
35	99.5	407	807	945	1106	1267
	49.7	50.9	50.4	39.5	24.1	17.6
43	13.7	136	175	273	392	—
	6.9	16.9	10.9	11.4	7.1	—
47	—	58	122	218	Perished	Perished
	—	7.2	7.6	9.1	—	—

To allow a more convenient study of the figures given in this table, curves are here added, showing the total production of chlorophyll (Fig. 1), and also the increase in chlorophyll under the influence of temperature during one hour's exposure (Fig. 2).

From them we see that the total production of chlorophyll increases as the exposure is prolonged, and attains its maximum after 3 days at 26° C. So the process of greening is in general a slow one, and in it the time factor plays an important part.

The influence of temperature is expressed in the ordinary form of curves, showing an optimum temperature of 26° C., except in the case of 8 hours' exposure to light, when 30° C. is found to be the optimum temperature. From this we must conclude that the optimum temperature does not depend upon the time of exposure, a fact most important from the theoretical point of view.

The rate of chlorophyll production also depends on the duration of exposure to light and the temperature. Except for the exposure of 8 hours with the optimum temperature of 30° C., in all other cases the maximum hourly production takes place at 26° C. So that here, too, the optimum temperature remains constant.

The difference is expressed in the fact that the hourly production

of chlorophyll attains its maximum after 8 hours' exposure to light and falls off with longer exposure. This is especially clearly seen in curves showing the variation of the rate of chlorophyll production with the duration of exposure to light (Fig. 3). Below 4° C. the rate

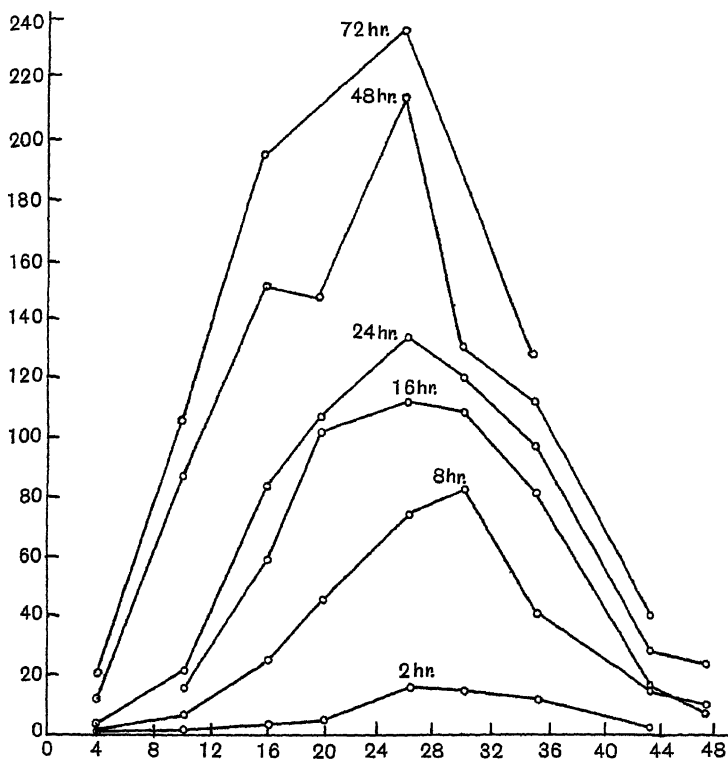


Fig 1. Curves showing the total production of chlorophyll at different temperatures after 2, 8, 16, 24, 48 and 72 hours of exposure of the seedlings to light. The abscissae show the temperature in degrees Centigrade; the ordinates, the quantity of chlorophyll in cg. per kg. fresh weight of leaves.

of production is accelerated with longer exposures and attains a maximum after 72 hours. At 10° C. the maximum is reached after an exposure of 48 hours, at 16 and 20° C. after 16 hours, and at 26 and 30° C. after 8 hours' exposure. At 35 and 43° C. the maximum is also reached after 8 hours' exposure. But if we consider that the temperature of 43° C. is near to the critical one at

which the seedlings begin to die, the figures for this temperature must be recognised as demanding reconsideration. In any case the increase in the rate of chlorophyll production with time, the temperature remaining constant, cannot be doubted.

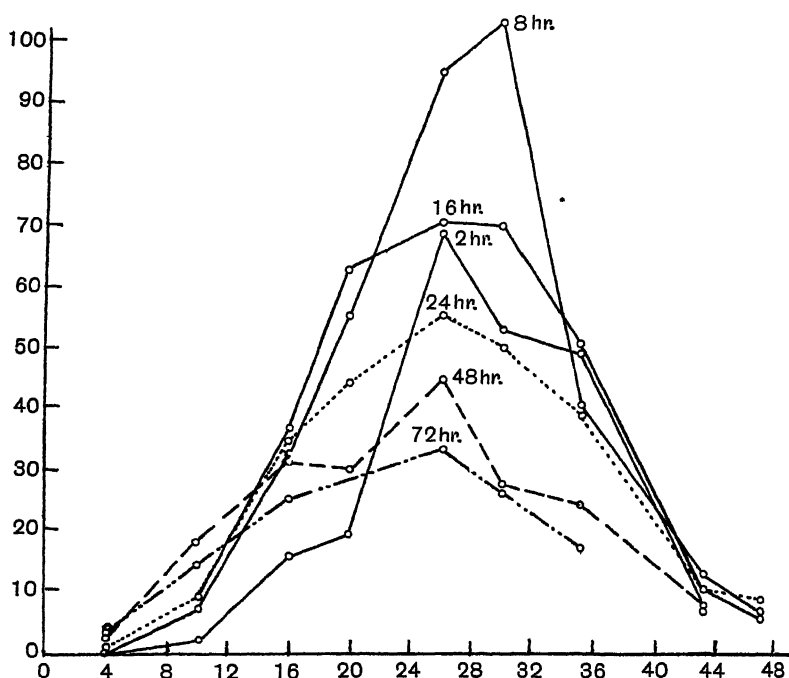


Fig 2 Curves showing the production per hour of chlorophyll at different temperatures after 2, 8, 16, 24, 48 and 72 hours of exposure of the seedlings to light. The abscissae show the temperatures in degrees Centigrade; the ordinates the quantity of chlorophyll in mg. per kg. of fresh weight of leaves.

If we now determine the temperature coefficients for the rate of production of chlorophyll, we shall obtain the following figures for the different times of exposure at different temperatures.

Temperature coefficient (Q_{10}) of the production of chlorophyll in 1 hour

Temp. ° C.	Duration of exposure of seedlings to light					
	2 hr.	8 hr.	16 hr.	24 hr.	48 hr.	72 hr.
4-14	8.8	15.4	—	7.0	5.4	4.7
10-20	4.7	8.0	6.5	5.0	1.6	—
16-26	4.1	2.9	1.9	1.5	1.4	1.3
20-30	2.6	1.8	1.1	1.1	0.9	—

These figures show that, with the same time of exposure, the temperature coefficient falls regularly as the temperature is raised. Also for the same increment of temperature, we observe a fall as the time of exposure is prolonged, and this is very regular for the intervals 16–26° C. and 20–30° C. In intervals of lower temperatures (4–14° C. and 10–20° C.) the increase in the temperature coefficients with the increasing duration of exposure from 2 to 8 hours is very marked. For higher temperature intervals (25–35° C.) the temperature coefficient falls to less than 1, after so short an exposure as 2 hours.

As the accumulation of chlorophyll in etiolated seedlings has a very definite limit, the falling off of the rate of production with the increase in time of exposure, can be explained as being due to the approaching of the accumulated chlorophyll to this limit. The accumulation of the pigment, as a product of reaction, must be recognised as a limiting factor here, and this product remaining naturally lessens the rapidity of the reaction.

In our experiments we have obtained the maximum quantity of chlorophyll at 26° C. after 72 hours; it was 2.362 grm. for each kilogram of fresh weight of leaves. If we consider that the maximum quantity of chlorophyll in adult leaves of wheat does not surpass, or only by very little, 4 grm. per kg. of fresh weight, the quantity of 2.362 grm. obtained by us can be, without serious error, recognised as the maximum quantity that young seedlings can accumulate at all.

From this figure we can calculate after how many hours of uninterrupted illumination the limit quantity of pigment at different temperatures could have been accumulated, supposing there to have been no retardation, and supposing the rate of production of chlorophyll to have remained at the maximum point for each temperature. This calculation gives the following figures:

° C			hr.
4	787
10	130.5
16	64.5
20	37.5
26	25.0
30	23.1
35	46.4

Thus, if there were no retardation, the shortest time for the limit

quantity to accumulate would be 23 hours, this taking place at a temperature of 30°C . at the maximum rate of production for this temperature. A figure, 25 hours, near to this minimal one, occurs at a temperature of 26°C . From this we can conclude that the optimum temperature for the accumulation of chlorophyll is that where the

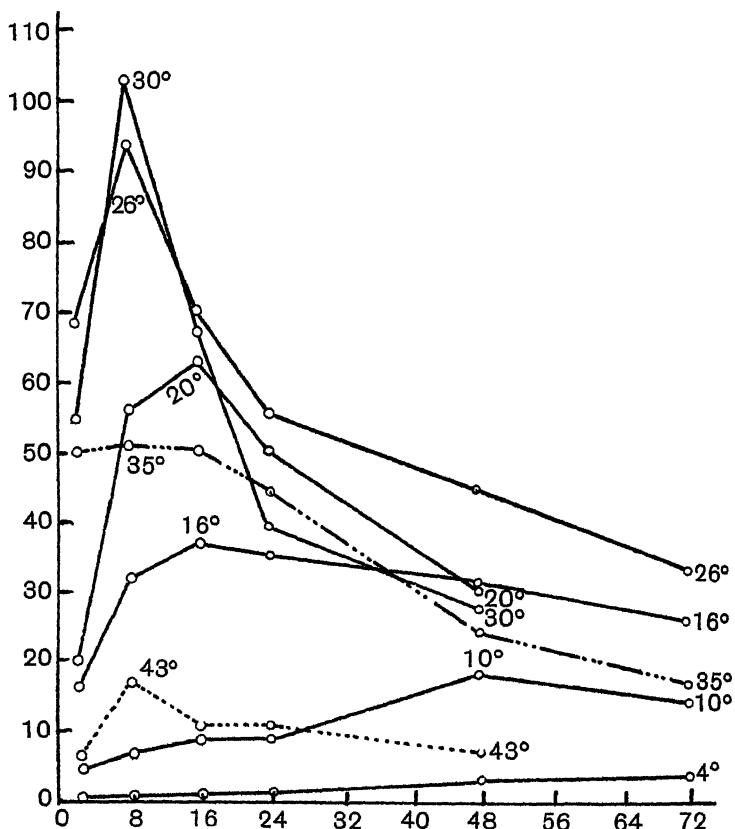


Fig 3 Curves showing the changes in the hourly production of chlorophyll depending on the length of exposure to light at different temperatures. The abscissae show the duration of exposure, in hours, of the seedlings to light; the ordinates show the quantities of chlorophyll in mg. per kg. of fresh weight of leaves.

velocity of reaction is greatest, and in our experiments this lies between 26 and 30°C . In practice, as we have shown, the rate of production of chlorophyll diminishes as the time of exposure is prolonged by reason of the retardation due to the accumulation of the pigment. This retarding effect begins already at a temperature of

10° C. after 48 hours' exposure, as can be seen in Fig. 3. As the temperature rises it becomes more marked, and its temperature coefficient can be determined for different temperatures.

In the following table we give the quantities of chlorophyll which could have been accumulated if the hourly production of pigment, having attained its maximum rate for certain temperatures, had remained constant; from these we deduct the quantities actually found, and the difference between these two will give figures showing the retardation effect. In the following table these last figures appear as denominators. We see from these figures that until a temperature of 30° C. is reached, the retardation increases with both temperature and time.

Quantities of chlorophyll calculated from the maximum rate of production in mg. per kg. of fresh weight

Temp. ° C.	Duration of exposure of seedlings to light			
	16 hr	24 hr	48 hr	72 hr.
10	—	—	872 10	1360 -250
16	581 ±0	853 -18	1171 -299	2630 -679
20	1005 ±0	1187 -123	2591 -1242	4201 ?
26	1512 -390	2268 -945	4536 -2399	6804 -4442
30	1635 -563	2452 -1253	4902 -3007	7352 ?
35	814 -7	1221 -276	2442 -1336	3663 -2306

Retardation has appeared at 10° C. after 72 hours' exposure, at 16 and 20° C. after 24 hours, at 26 and 30° C. after 16 hours, and increases with the duration of exposure to light. This is readily explained by the acceleration of chlorophyll production with rise of temperature; the more quickly the chlorophyll forms, the earlier will the retarding influence of the product of the reaction appear, and its value will be a simple function of its quantitative limit.

If we try to use the figures for the determination of the temperature coefficients of the retarding action, we obtain for the temperature intervals 16-26° C. and 20-30° C. the following values:

Temp. ° C.	Coefficient of retarding effect		
	after 24 hr.	after 48 hr	after 72 hr.
16-26	52.5	8	6.5
20-30	10.1	3	—

These quantities are much higher than the coefficients of chlorophyll production for the same intervals of temperature and the same duration of exposure to light. We must also consider the important fact of the lessening of the retarding action with rise of temperature and prolonged exposure to light. From this standpoint, the retarding, being the result of the accumulation of the product of reaction, presents a process allied to the process of accumulation, and follows the same laws concerning temperature and time. The difference consists only in the higher temperature coefficients of retarding, compared with the coefficients of chlorophyll accumulation. This fact has already been noted in the literature, and it may be possible that we meet here with facts of a general character. So, though the absolute quantitative expression of retardation increases with temperature, its rate, on the contrary, falls.

From all this, we can deduce that the increase in the rate of chlorophyll production with rise of temperature is closely bound up with the diminution in rate of retardation. Until now we have considered the influence of temperatures from 2 to 30° C., that is, in the interval finishing at the optimal temperature at which the limit quantity of chlorophyll accumulates in the shortest time. At temperatures lower than the optimal more time is needed to attain this quantity, but it is always ultimately reached.

We find quite a different set of phenomena for temperatures higher than the optimal one. If the same laws governed the accumulation of chlorophyll at these temperatures, then the limit quantity of chlorophyll would be accumulated in a shorter time as the temperature rises. So, if at 26° C. the maximum is reached after 72 hours, at 30° C. it ought to be reached sooner, and at 35° C. sooner still. But in reality, at temperatures higher than the optimal one, we observe a general fall in the rate of chlorophyll production for all times of exposure to light, beginning with the shortest of 2 hours. At the same time, the quantity of chlorophyll continues, even at these temperatures, to increase as the exposure is prolonged, which is also very significant. At temperatures higher than the optimal one, the accumulation of chlorophyll, as can be seen on the table of curves, becomes slower and slower with the rise of temperature; but the curves maintain the same general character as for the optimal temperature. Each of these curves approaches some limit of pigment quantity, but this limit becomes smaller with rising temperature.

This point is seen particularly clearly by comparing the curves

at temperatures of 30, 35 and 43° C. with the curve for a temperature lower than the optimal one, 16° C. for example (Fig. 4). At temperatures higher than the optimal one, we meet not only with the

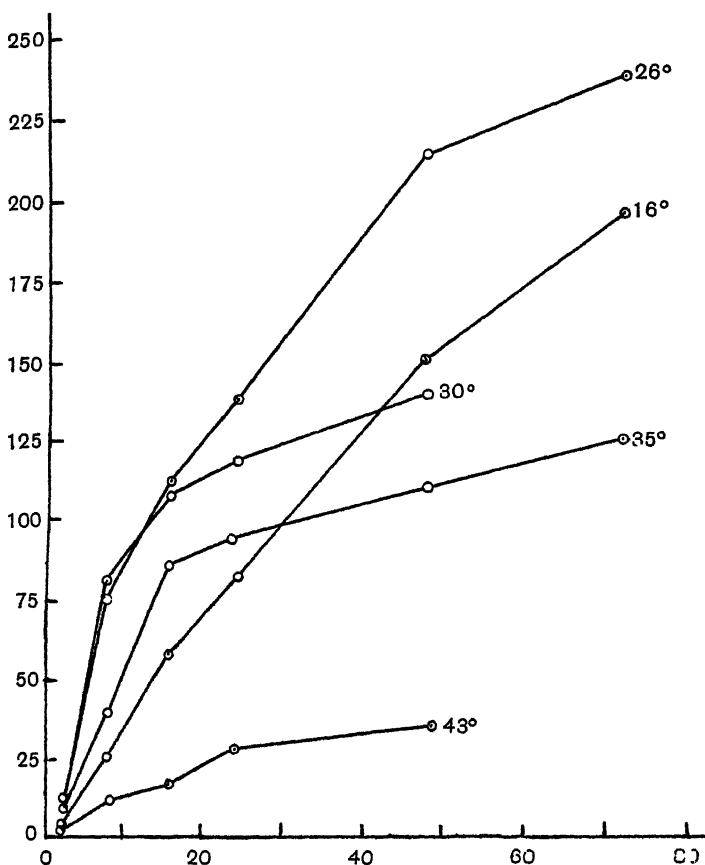


Fig. 4. Curves showing the increase in production of chlorophyll at temperatures of 16, 26, 30, 35 and 43° C., depending on the time of exposure of the seedlings to light. The abscissae show the time of exposure in hours; the ordinates the quantity of chlorophyll in mg. per kg. of fresh weight of leaves.

normal retardation due to the accumulation of the product of reaction, but with yet another factor which diminishes the total quantity of pigment that can be produced. The most probable explanation of this phenomenon is either that the chlorophyllogen formed at high temperatures is further transformed into a colourless substance, or

that the leucophyll changes not only into chlorophyllogen, but also into other substances.

In either case, we should have two reactions proceeding simultaneously, the accumulation of chlorophyll being proportional to the difference in rate of the two reactions. Until the temperature of 43° C. is reached, the rate of chlorophyllogen formation is greater than the rate of its decomposition and the result is a slow accumulation of chlorophyll; at still higher temperatures, the rates of the two reactions approach each other, and the plants cease to accumulate chlorophyll. Unfortunately the seedlings will not support temperatures of 48° C. and a little higher, hence we cannot determine exactly the maximum temperature at which the accumulation of chlorophyll completely stops. In any case for wheat this limit is near to 48° C.

DISCUSSION OF RESULTS AND CONCLUSIONS

The facts just stated provide material for some general considerations.

Temperature, as a factor which has a direct influence on the rapidity of chemical reactions in the organism, has especially attracted the attention of investigators, because its action in biological processes is very complicated. The quite general biological law, expressed by a curve with an optimal point, has been analysed many times, especially since Blackman attracted attention to the rôle of the time factor and came to the conclusion that no immovable optimal point exists in reality. In his opinion the chemical processes of the organism follow the rule of van t'Hoff, and the rate of reaction can be graphically expressed by a logarithmic curve. But in reality the rate of reaction falls off with time even at the same temperature, this fall occurring more quickly as the temperature increases. If it had been technically possible to determine the rate of reaction during the first moments, we should have obtained no optimal point. But as we are always too slow in our measurements, the result is the curve obtained, with an optimal point.

Concerning the curves without a clear optimal point, asymptotically approaching the horizontal line, they are obtained, according to Blackman, when one of the necessary factors is represented in insufficient quantity. This last conclusion, coinciding with the previously formulated law of the minimum of Liebig, has been experimentally verified; but a correction has had to be adopted. In the lack of some factor, the rate of reaction, having attained a certain limit, does not remain quite constant, as Blackman supposed, but increases

very slowly, approaching asymptotically a certain constant value. It is also necessary to consider that Blackman's conclusions are based on comparatively small experimental material concerning photosynthesis; and yet they have found an almost universal recognition. It may be for this reason that we do not meet with attempts at detailed analysis of the influence of temperature, complicated by the influence of time, in the course of biological processes.

In a résumé by Pütter on temperature and its influence on biological processes, the author draws especial attention to temperature coefficients. In agreement with Blackman, he thinks that the existence of an optimal point in the curve of temperature must be explained by the appearance of a secondary retarding process, obeying the same laws as the primary process, so far as temperature is concerned. The difference consists only in the fact that the temperature coefficients are much higher for the retardation process than for the primary one. Our experiments show that the greening, being a very slow process, is particularly convenient for the study of the time factor. The temperature coefficients fall with time, and, the quicker the course of the reaction, the more prompt is the fall in its rate. In this we quite confirm Blackman's conclusions.

So, the definition of the real rate of the reaction, and the exact determination of its temperature coefficient, demands a choice of temperature intervals, where the process develops slowly and where the influence of the duration of the experiment necessary for the measurement of the effect of the reaction, has a minimum effect. We have shown that the best interval of temperature for the greening of wheat is the interval from 0° to 10° C. In this interval are obtained the highest temperature coefficients, which can be determined after an exposure to light of 2 hours. At higher temperatures, the greening process accelerates in such a manner that even our shortest exposure of 2 hours becomes too long. So for the interval between 10 and 20° C., the temperature coefficient for an exposure of 2 hours is already much lower than for the interval of $4-14^{\circ}$ C. For the intervals of higher temperatures ($16-26^{\circ}$ C. and $20-30^{\circ}$ C.), a further lowering of the temperature coefficient, under an exposure of 2 hours, can be observed.

Now, according to Blackman's statement, the determination of the temperature coefficients at these temperatures demands a shortening of the time of experiment. But in our case, this assumption is not quite confirmed. The hourly production of chlorophyll, as can be seen from the given figures, increases as the time of exposure is

prolonged, at the same temperature. This increase is so considerable that it influences the temperature coefficients. So in our experiments, maximal temperature coefficients are obtained at temperature intervals of 4–14° C. and 10–20° C., with an 8 not a 2-hour exposure. We have to admit that this fact was quite unsuspected by us. As we have already said, the photochemical transformation of chlorophyllogen into chlorophyll is an instantaneous one and does not depend upon temperature. Our data can be attributed to only two consecutive dark reactions: the synthesis of leucophyll and the transformation of leucophyll into chlorophyllogen. These must be the slow reactions determining the real rate of greening and its dependence on temperature.

The increase in chlorophyll production with time, observed by us at constant temperature, must be attributed to the secondary action of light on the synthesis of leucophyll, or on its transformation into chlorophyllogen. Both reactions are endothermic, and, as our experiments show, do not take place at a temperature lower than 2° C., however long the seedlings are illuminated.

So the acceleration under the influence of light can be attributed to the increase in the quantity of energy due to its absorption by the greening plastids; and, in fact, with the raising of the temperature, the curve of the hourly production of chlorophyll mounts more steeply as the exposure is prolonged from 2 to 8 hours. The quantity of light absorbed, at the intensity used in this experiment, augments with the augmentation of the pigment. So the reinforcement of the secondary action of light, demonstrated by us, must be attributed to the absorbing action of the pigments formed in the plastids. The accelerating action of light does not depend in reality on time. Thus, at a temperature of 4° C., the hourly production of chlorophyll augments uninterruptedly for 3 days. At 10° C. the augmentation continues only for 2 days, and after that begins to decline; at higher temperatures this fall occurs yet earlier.

As we have already observed, this falling off is due to the approaching of the total chlorophyll quantity to a certain limit; that is why, as the chlorophyll accumulates more quickly, the falling off in rate occurs earlier. The following calculation shows that the diminution in this case is really determined by the quantity of chlorophyll accumulated. If we express in percentage the quantity of chlorophyll which must be accumulated to produce the beginning of the fall in the hourly production of pigment, taking as 100 the

maximal quantity obtained in our experiments (2.362 grm.), we obtain the following values:

° C.			%
10	37
16	21
20	42
26	32
30	35

From these values only one, corresponding to 16° C., differs sharply from the others; they are near enough to each other for an average value to be found, showing that the lowering of the hourly production of chlorophyll does not depend upon time, but only on the quantity of accumulated pigment, or more exactly, on the relation of this quantity to a limit quantity of pigment. The fall begins as soon as the quantity of accumulated pigment approaches 40 per cent. of the limit quantity, independently of temperature and time. If there had been no limit to the accumulation of chlorophyll, then the acceleration of the production of pigment under the influence of light would have continued uninterruptedly at all temperatures.

All this shows that both temperature and light influence the "dark" reactions of the synthesis of leucophyll, and its transformation into chlorophyllogen. We call these reactions "dark," because they can occur without the action of light. Being endothermic, they begin only at a fixed temperature. At temperatures lower than this, the quantity of energy absorbed by the seedlings under illumination is insufficient for these reactions. But at temperatures greater than this limit the absorption of the light energy accelerates the reaction.

Such is the course of the process with rise of temperature till a certain optimal point is reached. Our experiment shows that, with a certain constant intensity of light, the position of the optimal point remains unchanged and independent of the time of illumination of the seedlings. The optimal temperature can be defined as the one at which the limit quantity of chlorophyll is accumulated in the shortest time.

At all temperatures higher than the optimal one, the character of the phenomenon changes essentially. This change concerns, first of all, the limit quantity of pigment which becomes smaller with the raising of the temperature. We explain this diminution by the appearance of a new reaction resulting in the destruction of chlorophyllogen. The rate of accumulation of chlorophyll under these con-

ditions will be proportional to the difference between the rate of synthesis of the chlorophyllogen on the one hand and its destruction on the other.

The reaction governing the destruction of the chlorophyllogen depends also upon temperature, and the rate of this reaction can become so great that all the pigment formed will be decomposed. The temperature at which the rate of pigment destruction becomes equal to the rate of its synthesis can be called the maximal temperature. In our experiments we have not quite attained this point, but we have got sufficiently near it not to doubt its existence.

The destruction of pigment at higher temperatures, accompanying its synthesis, results in the lowering of the limit quantity of chlorophyll, compared with that produced at the optimal temperature. So, to the characteristics already stated of the optimal temperature must be added the fact that, at the optimal temperature the quantity of pigment attains in the shortest time the maximum possible for the given species. At temperatures lower than the optimal one, the accumulation of this maximal quantity is possible, but the process takes longer and the time steadily increases as the temperature falls. On the other hand, at temperatures higher than the optimal one, the accumulation of this maximal quantity is quite impossible, because the destruction of pigment going on at the same time lowers the total limit quantity.

The results of our experiments are not yet sufficiently complete or numerous to explain all the aspects of the influence of temperature on the greening processes. Thus, for example, the question remains unsolved as to which of the dark reactions, the synthesis of leucophyll or its transformation into chlorophyllogen, is the slower process, thus controlling the final result, the rapidity of greening. In the same way we lack the necessary data for our assumption as to the destruction of chlorophyllogen at temperatures higher than the optimal one; it may be possible that at these temperatures, leucophyll changes directly into some new compound.

We shall be able to make a complete analysis of the influence of temperature upon the greening process, only when we can ascertain sufficient facts to determine the rate of each of the reactions involved. But the knowledge we have obtained already permits of the statement that the principal, and perhaps the only inevitable retarding influence for the reaction of the organism, is the accumulation of its products. Under this influence the rate of the reaction falls off with time if the temperature is constant. But this "normal" retardation,

as it can be called, has no direct relation at all to the optimal temperature. The existence of an optimal point is conditioned by the appearance of another reaction, occasioning a direct lowering in the production of the given substance. In our case, the optimal temperature for the synthesis of chlorophyllogen is at the same time the minimal one for the new reaction, which changes the pigment into a colourless compound. As this new reaction is accelerated with rise of temperature, the total production of chlorophyll will fall, independently of the duration of the experiment, under the influence of temperature only.

Our experiments show, in fact, that the position of the optimal point of temperature remains unchanged, though in the experiment great variations in time had taken place. So the idea of a temperature optimum has a quite definite sense, which gives a reason for calling it the physiological optimum. Our considerations show that, physiologically, that temperature must be called optimal at which the physiological process is most rapidly accomplished, and at which the accumulation of substances characterising this process, attains a maximum in the shortest time.

The physiological optimum of temperature is the turning point at which appear new chemical reactions, changing fundamentally the course of the process, and resulting in the quantitative decrease of the products by which it is characterised. This is the reason why the physiological optimum has a great biological importance, as the expression of adaptation of the plant's chemical apparatus to the temperature conditions of the environment.

SUMMARY AND CONCLUSIONS

1. The greening process of etiolated wheat seedlings takes place within definite limits of temperature, beginning between 2 and 4° C., attaining its maximum rate between 26 and 30° C., and ceasing at a temperature near to 48° C.

2. The position of these cardinal points does not depend upon the duration of the seedlings' exposure to light.

3. The optimal temperature point is characterised by the accumulation of a maximal quantity of chlorophyll in the shortest time. At temperatures lower than the optimal one, the accumulation of the maximal quantity of pigment, which is the limit quantity for the given species of plant, demands more time as the temperature is lowered. At temperatures higher than the optimal one, the limit

quantity of chlorophyll diminishes and falls to zero as the maximal temperature is approached.

4. This relation of the greening process to temperature rests on the influence of this factor on the synthesis of leucophyll, and on its transformation into chlorophyllogen, since the transformation of chlorophyllogen into chlorophyll is a purely photochemical reaction, not depending upon temperature.

5. In the temperature interval from 2 to 30° C. the increase in the quantity of chlorophyll in a unit of time first grows and then falls with the length of time the seedlings are exposed to light at the same temperature.

6. The increase in the production of chlorophyll during the first period of exposure to light is conditioned by the secondary influence of light on the reactions of chlorophyllogen formation. As these reactions are endothermic, the absorption of light by the greening plastids augments the quantity of energy, and helps in this way the acceleration of the reactions.

7. The diminution in the production of chlorophyll during the following period of exposure, is conditioned by the retardation due to the accumulating mass of pigment remaining in the sphere of reaction. The fall in the production of chlorophyll in a unit of time begins from the moment when the quantity of accumulated pigment attains approximately 40 per cent. of its limit quantity, independent of temperature. That is why, the more rapid the accumulation, the earlier will appear the fall in the rate of production.

8. At temperatures higher than 30° C. a general fall can be observed in the production of chlorophyll, the quantity being smaller than that produced at the optimal temperature, even for the shortest exposure of seedlings to light.

9. This diminution of the total chlorophyll production at temperatures higher than 30° C. is probably conditioned by the appearance of a new reaction; under its influence is obtained from leucophyll, instead of chlorophyllogen, another uncoloured substance, or perhaps the chlorophyllogen already formed is decomposed.

10. The new reaction which diminishes the production of chlorophyll, begins at a temperature near to 26° C.; as the temperature rises its rate increases, and at the maximal temperature becomes equal to the rate of the chlorophyllogen synthesis. The production of this pigment, and with it that of chlorophyll then falls gradually to zero.

APPENDIX

ON THE SPECTROCOLORIMETRIC METHOD

The principle of this method is the same as that of colorimetry. The colorimetric quantitative determinations are based on the selective absorption of light by the solution; this absorption augments regularly with increase in concentration of the solution or the thickness of the layer through which the light is passed.

The analytical work consists in the visual comparison of the strength of light absorption by a solution of known concentration, with that of the solution which has to be analysed. In the practice of colorimetry the light absorption of both solutions is equalised by the help of the necessary augmentation or diminution of the thickness of the layer of one of the solutions, generally the experimental one, whilst in the standard solutions the thickness is kept always the same.

As light absorption shows a direct proportion to the number of molecules of the absorbing substance, the concentration C , of the solution to be analysed, can be expressed by the following formula:

$$C_1 = C_0 \frac{h_0}{h_1},$$

in which C_0 represents the concentration of the standard solution, h_0 its layer thickness and h_1 the layer thickness of the experimental solution at the moment when the light absorption of both solutions is equal.

If in an ordinary colorimeter, as for example, that of Dubosque, the eyepiece is replaced by a direct vision spectroscope in order to obtain a comparable absorption spectrum of each solution, the comparison of light absorption in different parts of the spectrum becomes possible.

In practice the work consists in comparing the intensity of certain selected absorption bands; in the case of quantitative chlorophyll analyses, the first, deeper absorption band in the red part of the spectrum of the alcoholic solution between $\lambda 670\mu\mu$ – $\lambda 650\mu\mu$ can be used.

In augmenting or diminishing the layer thickness of the solution which is to be analysed, we determine the moment when this absorption band attains the same intensity in the two absorption spectra. As light absorption is proportional in each part of the spectrum to the number of molecules of the absorbing substance, the

concentration of chlorophyll C_1 in the analysed solution will be determined by the same formula:

$$C_1 = C_0 \frac{h_0}{h_1},$$

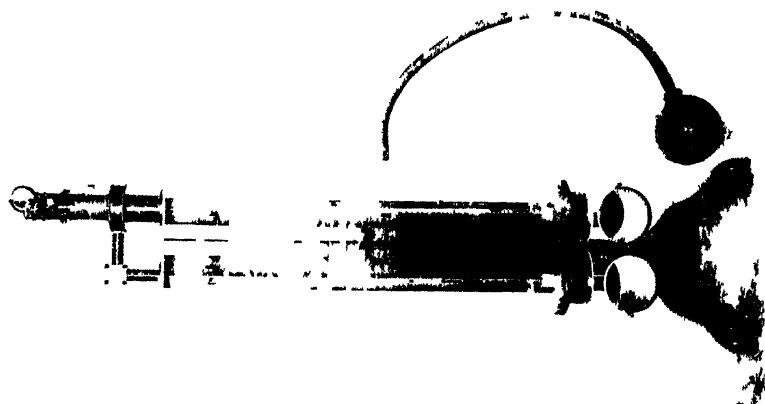
in which C_0 expresses the concentration of chlorophyll in the standard solution, h_0 the layer thickness of this solution, and h_1 the layer thickness of the analysed solution at the moment of equal intensity of the first absorption band in both spectra.

The study of the absorption spectra demands a spectroscopic examination of the pigment solutions of varying layer thickness; that is why it has been necessary to construct a special apparatus with a vessel for the solutions to be examined in which the thickness of the layer can be altered as desired. Such an apparatus can be easily adapted for comparative determinations, if a second vessel is added for the standard solution.

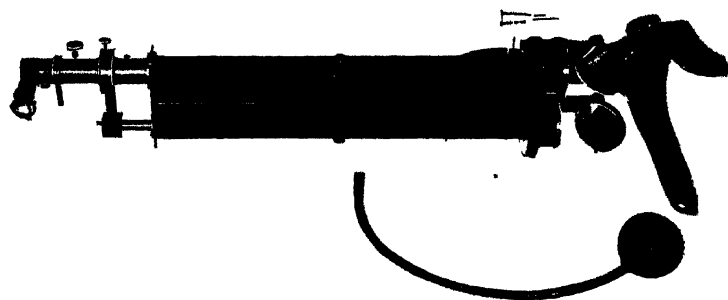
The first spectrophotometer based on this principle was constructed by us in 1907 and is described in the paper "La concentration du pigment vert et l'assimilation chlorophyllienne" (*Rev. gén. de Bot.* t. 20, 1908). The solution to be analysed was placed in this apparatus in a horizontal tube in which the layer thickness of the liquid could be varied by means of a screw. The standard solution was poured into a glass vessel with parallel sides and the whole apparatus adapted for the use of a big spectroscope.

The imperfection of this arrangement and the necessity to use a big volume of solution for analysis soon necessitated a modification in its construction. The new model was constructed with the assistance of N. A. Monteverde and is described in the paper "On the application of the spectrophotometrical method for quantitative analysis in the study of the accumulation of chlorophyll, xanthophyll and carotin in the plant" (*Russian Bull. of St Petersburg. Acad. of Sci.* 1913).

This model was afterwards perfected and constructed in 1927 in its last form by the firm "Goldberg und Sohne" (Berlin Potsdamer Str. 7). In its general plan the apparatus resembles the colorimeter of Wolf, but instead of short cylinders for the reception of the compared solutions tubes, 30 cm. high and 0.8 cm. thick, have been used, with mm. marked for determination of the layer thickness of the solutions. The standard liquid is poured into one of these vertically placed tubes and its layer thickness regulated by a glass cock. The liquid to be analysed is placed in the other tube; its layer thick-



Phot. 1 (left) Spectrocolorimeter with doors shut
 Phot. 2 (right) Spectrocolorimeter with open doors to show the vertical glass tubes for the standard solution (left side) and solution to be analysed (right side)



LUBIMENKO—THE INFLUENCE OF TEMPERATURE ON THE RATE OF ACCUMULATION OF CHLOROPHYLL IN ETIOLATED SEEDLINGS

ness can be either diminished by a glass cock, or augmented by a rubber balloon. (See the photographs, 1 and 2, of this apparatus on Plate II.)

Instead of a big spectroscope a modern microspectroscope of Zeiss or Leitz has been used. Our apparatus has in the upper part a metal tube, to which is attached, just as a microscope tube is, the under part of a microspectroscope. The slit of the microspectroscope is just over the tube containing the solution to be analysed, which allows of the direct observation of the chosen absorption band in the solution.

In order to obtain a comparable spectrum, the microspectroscope, as we know, has a round side opening illuminated by a small mirror. A small vessel with flat sides is fixed before the opening by two metal springs. We unscrew these and the mirror, and turn the microspectroscope so that its side opening comes opposite the short horizontal tube of our support. In this tube has been fixed a total reflection prism so that the ray of light, which has passed through the tube with the standard solution, is directed into the side opening of the microspectroscope; in this manner we obtained a comparable absorption spectrum of the standard solution. During the chlorophyll analyses, in both spectra, the same first absorption band can be observed, between $\lambda 670 \mu\mu$ – $\lambda 650 \mu\mu$ and it now remains to equalise the intensity of this band in both spectra, which is accomplished by the help of certain modifications of the layer thickness in the experimental solution.

Under each of the two vertical tubes of solution is fixed an ordinary concave mirror and over it an iris diaphragm serving to regulate the illumination of both liquids.

As a source of light an ordinary electric lamp of 30 or 50 candle power was employed, placed under a cover of white paraffined paper, helping to secure a regular diffused light.

The work is best done in a dark room or a dark corner where the eye is not irritated by another light. It is also advisable to protect the eyes from the lamp by a shade, so that only the light passing through the solutions may reach the eye.

To equalise most exactly the intensity of the absorption bands, the concentration and layer thickness of the standard solution must be chosen in such a way that the selected band may be found within the limit of clear view. The band must be as feeble as possible, like a light shadow, but still the eye must be able to distinguish it easily and exactly.

Our experiments have shown that, for chlorophyll analyses, a standard solution of 0.001 to 0.002 mg. of crystalline ethylchlorophyllide in 1 c.c. of alcohol is sufficient. Such a solution placed in the tube shows hardly any green colour if observed from the side, yet in a layer of 5 cm. it shows already a clear absorption line, its intensity being sufficient for analysis.

In analyses of carotin and xanthophyll the concentration of the standard solution must contain, in a layer thickness of 5 cm., not more than 0.001 mg. of crystals in 1 c.c. of solvent liquid.

For the determination of phycoerythrin in red algae, our standard solution contained 0.013 mg. dye substance in 1 c.c. of water with a layer thickness of 5 cm. As our tubes are 30 cm. high, the experimental solution can be 6 times feebler than the standard one; so it can contain 0.00017 mg. of chlorophyll, carotin and xanthophyll and 0.0025 mg. of phycoerythrin in 1 c.c. of solvent.

The absorption of light by the solution consists of two factors: the absorption of the dissolved pigment and that of the solvent; for this reason it is advisable to choose for colorimetrical analyses a concentration of the experimental solution which is as near as possible to the standard concentration (in order that the layer thickness of both solutions may not present great differences). Only under these conditions will the difference of absorption by the solvent in the compared solutions have no influence on the exactness of the determinations.

But in our apparatus, instead of varying the layer thickness of both solutions, we vary respectively the strength of illumination, using the iris diaphragm. A great exactness in the comparison of the absorption bands is only possible when the adjacent bright parts of the two spectra are illuminated with equal intensity. As too bright spectra diminish the sensibility of the eye, it is advisable to work with spectra whose brightness does not excite the eye too much, being yet sufficient for a clear perception of the absorption bands. It is very important in this case that the slit of the spectroscope should be sufficiently narrow, as, when the slit is broad, the limits of the absorption bands become less clearly defined. The best width of slit to choose is that at which the principle lines of Fraunhofer are clearly seen under illumination of the slit by diffuse daylight.

The intensity of both spectra is equalised by moving a mirror before the experimental solution is poured in, and then the brightness of the illumination is regulated for the spectrum of the standard solution as is desirable for the analysis. After that, the experimental

solution, which is generally weaker than the standard one, is poured into the tube, and when the intensity of its absorption band is very near that of the standard solution, the illumination and intensity of the parts of the spectrum of the experimental solution are equalised with those of the standard solution by the help of the iris diaphragm. Only after that will be determined the necessary layer thickness of the experimental solution at which its band becomes of equal intensity with that of the standard solution. It is advisable to adapt oneself, before the beginning of the experiment, to the new method, and to determine the magnitude of personal error, which has its source in the sensibility of the eye. This can be ascertained by preparing different portions of standard solution, of different, already known concentrations, by diluting the original solution. Our trials have shown that, in the case of normal eyes, the error does not exceed 1.5 per cent.

As is seen by this description, the spectrocolorimetric method enables quantitative analyses of dye substances to be made with great exactness; there is no necessity to isolate them from any admixtures, not only of uncoloured substances, but even of other pigments, if the absorption bands chosen for analysis do not coincide with each other.

Our experience has shown that the quantity of chlorophyll in ordinary alcohol solutions can be determined by this method with great exactness, without separating it first from the yellow pigments, and that certainly makes the work quicker and simpler.

In a mixture of chlorophyll and protochlorophyll, each of these substances can be determined separately; though both are green in colour, their first absorption bands occur in different parts of the spectrum; the chlorophyll band at $\lambda 670\mu\mu$ – $\lambda 650\mu\mu$ and that of protochlorophyll at $\lambda 640\mu\mu$ – $\lambda 620\mu\mu$.

As our method is a micromethod, the quantity of material which is demanded for analysis is quite small. For the determination of the chlorophyll quantity in normal green leaves, portions of 0.05–0.1 grm. of fresh weight are sufficient for us. A complete extraction of pigments is obtained by rubbing in a mortar with 10 to 20 c.c. of alcohol for 15 minutes.

Extractions obtained in this way must generally be diluted from 5 to 10 times.

In order to determine the content of xanthophyll and carotin in the green parts of the plant, one must necessarily separate them from chlorophyll. This is best done by precipitation with barium

hydroxide, which forms with chlorophyll an insoluble combination. After optical definition of the content of chlorophyll, a procedure generally lasting only one minute, the alcohol extract of pigments is treated with excess of barium hydroxide, all the pigments forming in that case a flaky precipitate. One may use for that a saturated solution of barium hydroxide, as free as possible from barium carbonate. After the precipitate has remained 6 to 12 hours in darkness, it must be passed through a filter and dried on the filter paper. Then the precipitate is treated with strong, preferably absolute alcohol, to extract all yellow pigments. To this alcohol extract is then added an equal volume of petrol ether and a few drops of water. Shaking in a separating funnel separates the liquid into layers: carotin goes into the upper one, and the xanthophyll remains underneath. As some carotinoids are insoluble in alcohol, the precipitate must be treated, after its treatment with alcohol, also with petrol ether.

The precipitate obtained by the action of barium hydroxide can also be dried first on filter paper and afterwards in the air, or in a desiccator in the darkness. By treating the dry precipitate first with petrol ether and then with alcohol, carotin can be separated from xanthophyll. This method takes longer, as the precipitate must in this case be dried. In this way the quantities of chlorophyll, carotin and xanthophyll can be determined with great accuracy in portions of 0.05–0.1 grm. of leaves.

If an expression of the quantities of obtained pigments in absolute units of weight is desirable, the standard solutions must be prepared from crystalline compounds. To obtain chlorophyll one can prepare ethylchlorophyllide by the method of Borodin-Monteverde worked out by Willstätter. Carotin and xanthophyll can also be prepared in crystalline form by the method of Willstätter.

Yet it must be recognised, that the obtaining of crystalline preparations of all three mentioned substances, and especially of chlorophyll, is very complicated, demanding the treatment of a large quantity of material.

But for biological analyses the relative determinations of the three pigments are in many cases sufficient; with a standard solution from the leaves of some plant, a grass for example, wheat being the best choice. 200 to 300 grm. of freshly gathered leaves must be dried in the air in the dark at 30–40° C. so as to keep their natural green colour. This store of leaves can then be kept in the desiccator in the dark for some months.

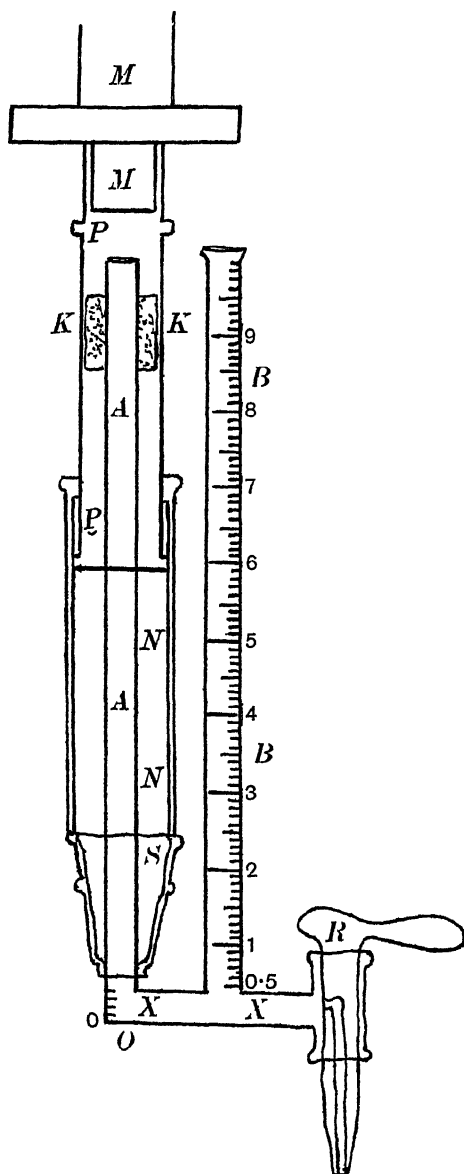


Fig. 5. Simple arrangement for spectrocolorimetric analysis with the aid of microscope and microspectroscope. For explanation of lettering see text.

A sample of dry leaves weighing from 1–2 grm. is rubbed in a mortar with strong alcohol until the pigments are completely extracted. The alcohol extract obtained in this way is divided into two portions; one of them serves as a solution for the preparation of the standard solution of chlorophyll, the other is treated in the way just described with barium hydroxide and serves for separation of carotin and xanthophyll and for preparations of mother solutions of these pigments.

For less precise analyses we have constructed a simpler model of spectrocoulometer. This apparatus consists of two glass tubes, *AA* and *BB*, joined by a third, *XX*, with a cock *R* (see Fig. 5). For its fixation can be used an ordinary microscope with an objective of low magnification, its lens having been previously taken away. The principal tube *NN* is taken out from the rack of the microscope and the tube *AA* of the apparatus is introduced in its place in the opening of the objective *S*. The upper end of the tube *AA* is centralised and fixed into the interior tube *PP* of the microscope by the ring of an ordinary cork *KK* fixed on the upper end of the tube *AA*.

Then the tube *NN* of the microscope is put into communication with the rack and the apparatus fixed so that the tube *XX* may lean on the table of the microscope. It is preferable to make the bottom *O*, of the tube *AA*, quite flat, in order to suppress as much as possible the irregular reflection of the light, penetrating into the tube *AA* through the opening in the table of the microscope. For the same reason is introduced into this opening a diaphragm, its opening being a little smaller than the diameter of the tube *AA*; the end *O* must be adjusted to this diaphragm when the tube *NN* of the microscope is definitely fixed. We use for our apparatus glass tubes of 5–6 mm. interior diameter and 18–20 cm. long.

The interior tube *PP* of the microscope is then pulled out so that the upper open end of the tube *AA* is placed lower than that of the tube *PP*; after that one fixes the microspectroscope into the tube *PP*, taking care that the lens of the microspectroscope does not touch the upper open end of the tube *AA*. One pours the liquid which has to be examined through the top open end of the tube *BB*, on which are marked the divisions (in mm.) serving to determine the layer thickness of the liquid. Generally the solution soon finds the same level in the tubes *AA* and *BB*; but if all parts of the metallic tubes are well polished air will enter the tube *NN*, as well as leave it, with insufficient promptness. That is why one must wait some time or put under atmospheric pressure the air in the tube *NN* by prudent

movements of the lower end of the microspectroscope. As we see, it is the solution in the tube *AA* that has to undergo the direct spectroscopical examination. The tube *BB* serves to determine the thickness of the liquid layer in the tube *AA* and also to augment or diminish the layer, which is easily done by simply adding some liquid through the upper end of the tube *BB* or by opening the cock *R* in order to let a part of the liquid flow away.

The length of the horizontal tube *XX* must be such that the cock *R* may come over the edge of the microscope stage.

It is clear that the addition of the tube *BB* in our apparatus doubles the minimal volume of the solution to be analysed, but the interior diameter of the tubes *AA* and *BB* is so small that the total volume of liquid employed does not exceed 15 c.c. for 20 cm. of layer thickness.

We pour the standard solution into an ordinary small glass vessel with parallel sides, fixed by an ordinary cork on a metal support, especially made for this purpose on the microspectroscope, opposite to the side opening, in order to obtain the comparable spectra.

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THE SPORANGIOPHORE OF *PILOBOLUS*

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(With 2 figures in the text)

ALTHOUGH *Pilobolus* has been extensively studied both by mycologists and by physiologists, there does not appear to be any clear account of the exact manner in which the sporangium is discharged. In this short note it is proposed to give an illustrated description of the development and dehiscence of the sporangiophore in *Pilobolus Kleinii* based on original observations.

Pilobolus appears almost invariably on the dung of most herbivorous animals. If fresh horse-dung balls are kept on moist filter paper in a dish and covered with a bell-jar, sporangiophores of *P. Kleinii* develop after a few days and then crops of mature sporangiophores are produced daily for a week or two.

DEVELOPMENT OF THE SPORANGIOPHORE

The non-septate mycelium of the fungus develops in the dung ball, germination in the first instance being induced by the relatively high temperature of the horse's intestine.

The sporangiophore primordium is first visible to the naked eye as a minute spherical orange bulb protruding slightly above the general level of the substratum. Microscopic examination shows that this bulb is cut off from the greatly enlarged end of a hypha, and that the spherical primordium is packed with minute globules of yellow oil (Fig. 1, *a*). This is the condition of the young sporangiophores in the forenoon.

During the course of the afternoon an intensely yellow branch grows up from the primary bulb (Fig. 1, *b*). If a horse-dung culture bearing *Pilobolus* is examined between 3 and 5 p.m.¹ these little yellow processes (about 2 mm. high) are very conspicuous on account of their brilliant colour. From this stage onwards the tip of the sporangiophore is markedly heliotropic (Jolivet, 1914; Parr, 1918).

Towards evening (6-8 p.m.) the tip swells up into a minute spherical bulb destined to become the sporangium (Fig. 1, *c*), and at

¹ The times referred to in this paper are in Summer time.

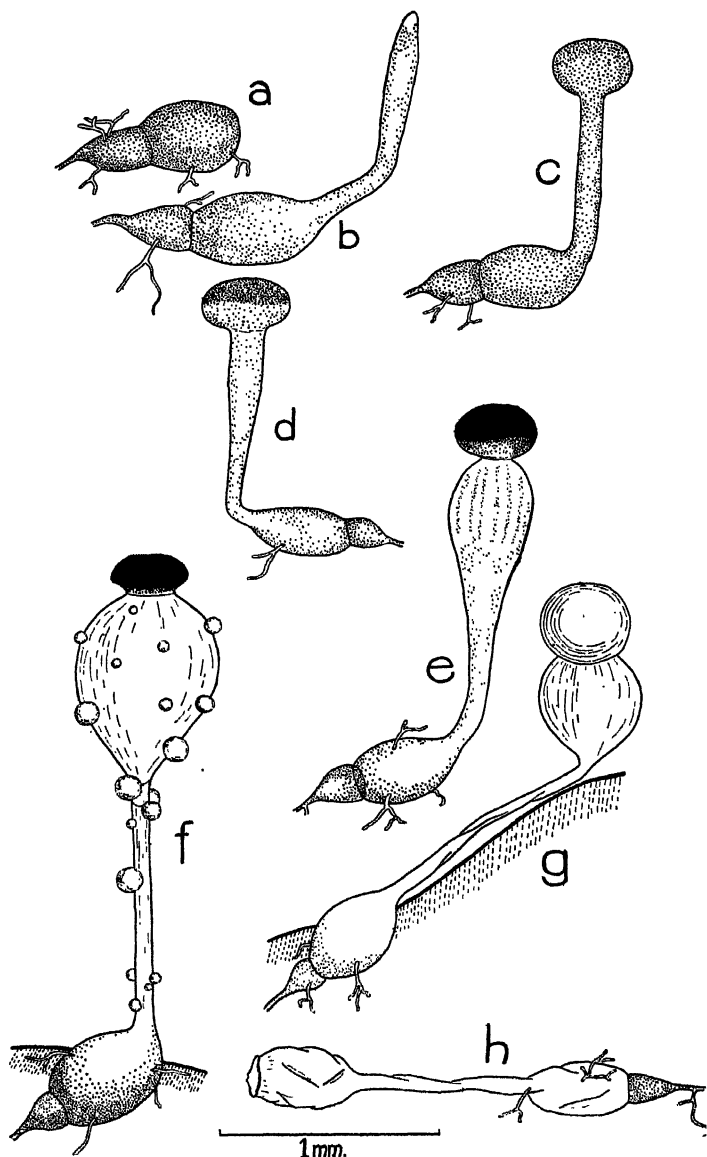


Fig. 1. *Pilobolus Kleinii*. *a*, spherical primordium stage found in forenoon; *b*, bright yellow process developed from the primordium (3-5 p.m.); *c*, swelling of the tip of the process to form the sporangium (6-8 p.m.); *d*, massing of the contents in the terminal swelling. Subsporangial swelling just beginning. Stalk becoming colourless (8-10 p.m.); *e*, black cap completely formed, stalk almost colourless, subsporangial region considerably swollen (about midnight); *f*, mature sporangiophore (8-11 a.m.); *g*, sporangio-phore a few seconds after dehiscence exuding a drop of liquid; *h*, the deflated sporangiophore some hours after dehiscence showing the circular line of dehiscence. All the figures are drawn to approximately the same scale.

this stage the oil drops giving the yellow colour to the sporangiophore are evenly distributed throughout.

As night approaches (8-10 p.m.) the oil tends to accumulate in the upper spherical swelling and the stalk region becomes more or less colourless. At the same time the wall of the upper part of the terminal swelling begins to blacken and the subsporangial region starts to swell. This condition is shown in Fig. 1, *d*.

Towards midnight the subsporangial swelling develops considerably and the black cap of the sporangium becomes fully formed, but the spores are still undifferentiated (Fig. 1, *e*).

In the early morning (6-8 a.m.) the sporangiophores are practically mature (Fig. 1, *f*). At this stage the subsporangial swelling is swollen to its full size, the stalk region is completely elongated and the spores are fully formed.

Throughout the development of the aerial portion of the sporangiophore minute drops of water are to be seen covering the surface, presumably exuded on account of the great hydrostatic pressure within the cell. These drops become particularly conspicuous and abundant on the mature sporangiophore.

Immediately before explosion the sporangiophore consists of (1) a lower bulb immersed in the substratum, (2) an erect stalk, and (3) an enlarged subsporangial bulb ending in a dome-shaped columella which protrudes into the sporangium. The stalk and the upper bulb of the sporangiophore contain a clear watery liquid except at the point where the stalk joins the bulb; here a conspicuous zone of oil is invariably found. This oil appears to the naked eye as a minute orange spot at the base of the subsporangial swelling and has been interpreted by Buller (1921) as an eye spot, the bulb itself being regarded as a lens focussing the light on the sensitive spot.

The sporangium wall is differentiated into a hard upper blackened region and a very thin non-resistant lower region. Before discharge this thin wall partially breaks down. Within the sporangium, and occupying the upper part of it, is a mass of yellow-orange spores completely hidden by the black cap. The remainder of the sporangium is occupied by clear mucilage. The structure of the mature sporangium is illustrated diagrammatically in Fig. 2, II.

Sporangium discharge occurs in the morning between 9 and 12 o'clock. The formation of the sporangiophore, from the spherical primordium until discharge occurs, therefore occupies just one day. At any time all the sporangiophores are at approximately the same stage in development. It is probable that the production of daily

crops of sporangiophores is connected with the periodic changes in light intensity associated with the alternation of day and night.

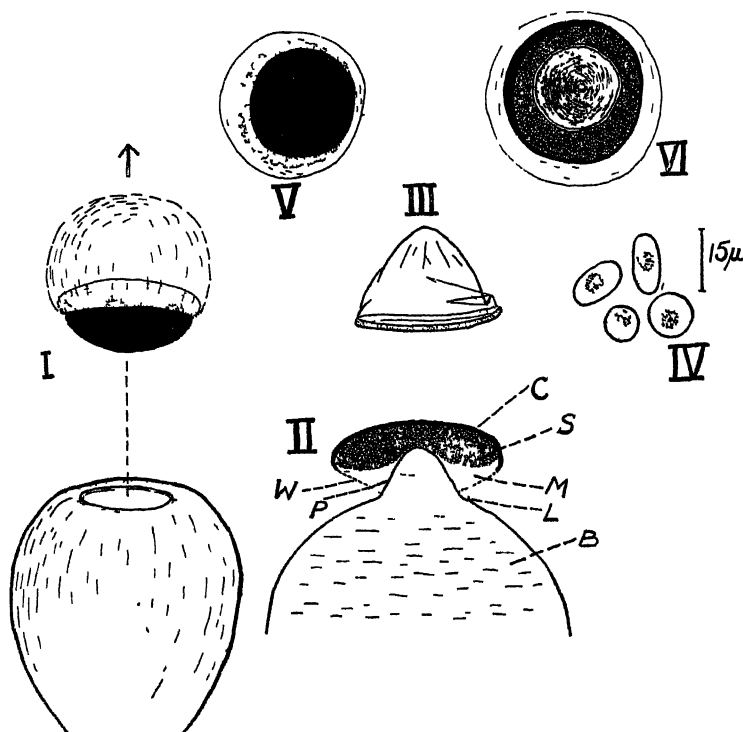


Fig 2. *Pilobolus Kleini*. I, diagram showing the supposed form of the projectile. Below is the contracted subsporangial bulb, above is the projectile consisting of a drop of liquid carrying an inverted sporangium. II, diagram of the sporangium. B, the subsporangial bulb; L, the line of dehiscence; P, the columella; W, the thin part of the sporangium wall; C, the black cap; S, the mass of spores; M, the clear mucilage; III, the columella dissected out from a discharged sporangium. IV, spores. V, surface view of the sporangium caught on a glass slide. In the centre is the black cap, fringing this is what remains of the thin region of the wall. The remainder is clear mucilage. No spores can be seen. VI, sporangium caught on a glass slide and viewed through the slide. In the centre is the hollow cone of the columella, around this can be seen a mass of spores and outside this mass the edge of the black cap. Outside this again is the clear mucilage.

DISCHARGE OF THE SPORANGIUM

The pressure inside the sporangiophore finally becomes so great that an explosion results, rupture occurring along a very definite line of weakness where the columella joins the subsporangial bulb. The elastic wall of the sporangiophore contracts almost instan-

taneously with a very audible click. The explosion takes place so quickly that it is impossible to follow the exact details at this stage. It is, however, the opinion of the writer that a small drop of liquid is ejected from the sporangiophore and on escaping tears off and carries away the sporangium. If sporangia are caught on a glass slide held several centimetres above a culture of exploding *Piloboli* it can readily be seen that the sporangium is accompanied by a considerable amount of clear watery liquid presumably derived from the subsporangial bulb. The amount of this water appears to be about twice the volume of the sporangium itself. The sporangia caught thus on a slide are always the same way up *with the black cap looking downwards*, the mucilage and the rim of the columella being stuck to the surface of the glass (Fig. 2, V and VI). It is clear that the sporangium on leaving the sporangiophore, and before it hits an object held above it, must in some way be turned upside down. The manner in which this inversion takes place may be visualised in the following way. The columella probably begins to tear away from the sporangiophore at a point on the circumference of the line of dehiscence and the tear rapidly spreads. Through the aperture thus produced water exudes forming a drop which, as it grows, increases the tear. This drop is moving with great velocity and as it rounds off, in separating from the sporangiophore, tears away the sporangium completely, so that in the projectile the sporangium is at the bottom of the drop with the black cap undermost forming an unwetted base to the drop (Fig. 2, I). The time occupied from the initial break in the dehiscence line to the separation of the projectile occupies only a very small fraction of a second.

Immediately on the discharge of the sporangium the stalk region of the sporangiophore loses its rigidity and the bulb, which has contracted to about half its original volume, is brought down to the level of the substratum. The bulb is, however, still full of liquid, part of which is soon squeezed out, as a small drop, by further slow contraction of the wall (Fig. 1, *g*). After a short time the old sporangiophore becomes completely deflated (Fig. 1, *h*).

Microscopic examination of the sporangiophore immediately after explosion shows that the line of dehiscence is a very perfect circle.

The discharged sporangia exhibit certain interesting features; these are illustrated in Fig. 2, III, V and VI. The mucilage of the sporangium serves to stick it to whatever object it happens to strike. The black cap of the sporangium completely covers the spores and possibly protects them from the injurious action of light. The remains

of the unthickened region of the sporangium wall can still be identified in the discharged mass and the columella is a cap-like cone which may with care be dissected out. The number of spores contained within a single sporangium is very great.

Buller has found using *Pilobolus Kleinii* and *P. longipes* that the sporangia are shot to a maximum height of six feet and a horizontal distance of eight feet. With *P. Kleinii* the writer has found that sporangia may be shot to a height of 118 cm.

The ease with which this fungus may be obtained in quantity and the interesting features which it exhibits render it specially suitable for class work. An intensive study of the development of the sporangiphore and the discharge of the sporangium is both instructive and interesting for advanced students.

For microscopic examination the sporangiphore must be removed from the substratum, care being taken not to rupture the lower bulb which is partially immersed in the substratum. The sporangiphore must be mounted in water since glycerine causes immediate deflation.

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A NOTE ON THE RESPONSE OF GRAM (*CICER ARIETINUM* L.) SEEDLINGS TO ELECTRICITY

BY T. C. N. SINGH

(With 2 figures in the text)

PRELIMINARY experiments carried out in the Botany Department of Lucknow University showed a striking modification of the root system under the influence of weak electric currents. Healthy and uniform looking gram seeds were sown in batches of twenty in pots containing garden soil. When germination had occurred two pots were placed on rubber pads and one continuously electrified as shown

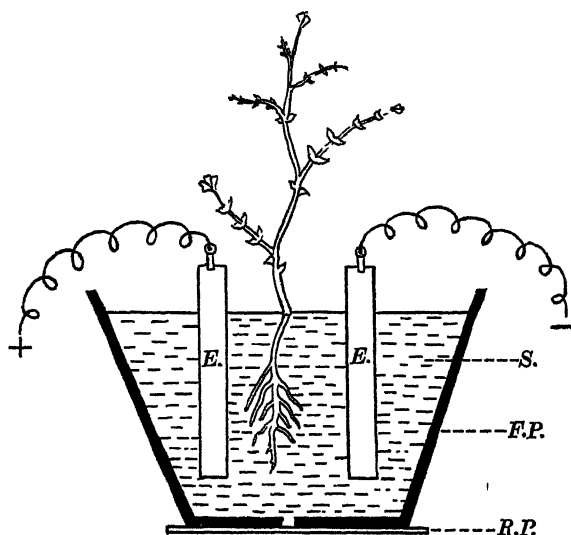


Fig. 1. Method of electrification. *E.*, electrode; *R.P.*, rubber pad; *F.P.*, flower pot; *S.*, soil.

in Fig. 1. Water was given to the cultures in equal quantities and under identical conditions. After thirteen days the thirteen surviving seedlings in the electrified pot had an average shoot length of 8.2 in. and an average root length of 2.5 in.; the corresponding numbers for the control pot being 8.5 and 9.0 respectively. In a second and third experiment more cautious watering prevented the death of so many seedlings in the electrified set. The treated roots again showed a similar stunting, but the shoots showed greater vigour than those

of the controls. A further notable difference in the two root systems was the abundant production of tertiary rootlets in the electrified culture and their entire absence in the other (Fig. 2 *a* and *b*).



Fig. 2. Two gram seedlings: *a*, from an electrified culture; *b*, from a control. The seedlings are shown bent for convenience. *P.R.* = primary root; *S.R.* = secondary and *T.R.* tertiary.

ON A *CHARACIUM* GROWING ON *ANOPHELES* LARVAE

BY M. O. P. IYENGAR AND M. O. T. IYENGAR

(With Plate III and 1 figure in the text)

IN connection with malaria research the second author has to examine large numbers of *Anopheles* larvae collected daily from several villages near Sonarpur in Lower Bengal. In the course of this work, many larvae were observed to show a greenish appearance which on microscopic examination proved to be due to a fairly dense growth of a species of *Characium* (Plate III, 2-5), often accompanied by a small number of young plants of an *Oedogonium* in a sterile condition. Several species¹ of *Characium* have been recorded as occurring on *Cyclops* and other minute crustaceans, as well as on Rotifers, but so far none have been reported on living mosquito larvae. *Oedogonium* does not appear to have been so far found growing on any living animal.

The *Characium* occupied practically all parts of the body of the larva except for the ventral surface where it was scanty or absent. There was a fairly rich growth on the back and flanks of the larva and the anal portion was often densely covered. The algae are associated frequently with certain Vorticellae, and the growth of these various epizoid organisms was often so considerable as to render the identification of the larva difficult. The algal growth seemed to hamper to some extent the freedom of movement of the larva, but the latter did not otherwise appear to suffer in any way.

The frequent occurrence of these algae on this particular substratum is not purely accidental, since other suitable substrata (*Pistia*, *Lemna* and other aquatics, as well as objects lying in the water) in the same ponds never bore these forms. The many larvae of the *Culex* mosquito and May-fly larvae present did not show any growth of the two algae, which were in fact restricted to the larvae of *Anopheles*. It is evident that they favoured a moving substratum

¹ J. Brunnthaler in Pascher's *Susswasserflora*, Heft 5, mentions the following six species of *Characium* as occurring on animals: *C. cylindricum* F. D. Lambert and *C. gracilipes* F. D. Lambert on *Branchipus vernalis*, *C. groenlandicum* P. Richter on Phyllopods, *C. Hookeri* (Reinsch) Hansgird on species of *Cyclops*, *C. Debaryanum* (Reinsch) De Toni on Crustaceans and *C. limneticum* Lemmermann on *Diaphanosoma*. Filarszky (2) records *C. saccatum* Filarszky and *C. setosum* Filarszky as occurring on *Branchipus stagnalis*.

and, among the available aquatic animals, the *Anopheles* larvae in preference to other larvae. By growing on an actively moving larva the algae secure better aeration than is afforded in a stagnant and consequently poorly aerated piece of water. The *Characium* riding on its ever active host is carried into different areas of the pond where fresh supplies of dissolved gases may be available. The cutaneous respiration of the *Anopheles* larva ensures a good supply of carbon dioxide. The frequent dense aggregation of the algae on the anal gills, where cutaneous respiration is greatest, is possibly due to the large supplies of CO₂ available, as well as to the nutriment derived from the excreta of the larva.

When the *Anopheles* larva comes to rest near the water surface, it floats horizontally with the dorsal side upwards and fully exposed to the sky, so that the algae receive plenty of sunlight. The presence of the alga in large numbers on the back and flanks of the larvae and its absence or scarcity on the ventral surface may be the result of the normally greater illumination of the former. The absence of the alga on the larvae of *Culex* and allied genera in the same water is perhaps due to their different habits. The larva of *Culex* does not float horizontally, but hangs downwards from the surface of the water. An alga will therefore not obtain as much sunlight when growing on a *Culex* or similar larva as when growing on an *Anopheles* larva.

The larva moults periodically under normal conditions once in every three or four days and with the old skin all the algae growing on it are shed. The larva, however, very soon becomes covered with a fresh coating of algae, whose growth is remarkably rapid, zoospores apparently being formed at short intervals. Plenty of empty cells of *Characium* from which the contents have already escaped are to be found on the living larvae. The zoospores often settle down on the empty walls and grow direct into new *Characium* plants (Plate III, 3 and Fig. 1, C, D, H), and it is not uncommon to find plants of a third generation growing on these latter (Fig. 1, J). This shows clearly that the time taken for the zoospore to settle down on the larva and grow into a new plant forming zoospores in its turn is much shorter than the interval between two moulting periods of the larva.

The larvae of the following six species of *Anopheles* served as hosts for the *Characium*: *A. vagus* Donitz., *A. subpictus* Grassi, *A. pseudojamesi* Strickland and Chawdury, *A. varuna* Iyengar, *A. hyrcanus* Giles and *A. barbirostris* v. d. Wulp.

The cells of the alga are pear-shaped, squat (Plate III, 4) or elongated, broadly rounded at the top and narrowed gradually below into a rounded base. They are attached by a very minute mucilage pad to the body of the larva. This pad is seen only on staining and careful examination under higher powers. The cells have a central nucleus and the chloroplast has a single pyrenoid. Division of the

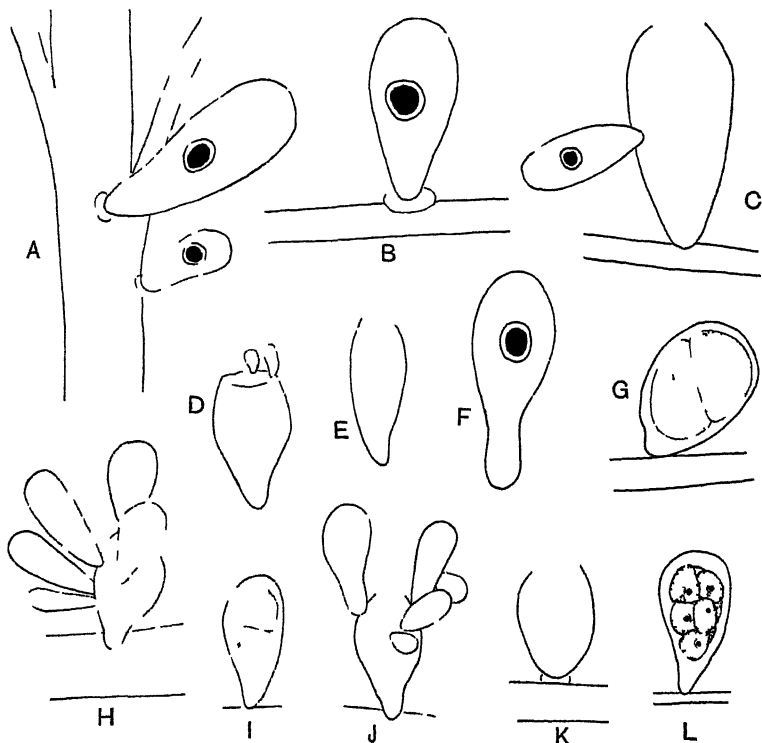


Fig 1 *Characium anopheles* sp. nov. A, two cells on the hairs of an *Anopheles* larva; B, a single cell, C, D, H, daughter-cells growing on empty cells, E, K, empty cells, F, a cell with a very broad base; G, I, division of cell contents into two, J, a colony of three generations, L, division of cell contents into eight Pyrenoids black A-C, F $\times 1000$, the rest $\times 638$.

contents is into 2, 4 or 8 parts which presumably escape as zoospores which have, however, not been directly observed. The contents escape by a clean rupture of the cell wall at the top, the empty mother wall appearing urn-shaped (Fig. 1, C, D, E, J, K). As already mentioned the contents often germinate on the empty walls and form colonies (Fig. 1, C, D, H, J). The dimensions of the fully grown



1



2



3



4



5

IYENGAR—ON A *CHARACIUM* GROWING ON *ANOPHELES* LARVAE

cells are $41-48 \times 22-30 \mu$, the smaller dimension being the width of the cell at its broadest portion.

The alga appears to be a new species which we propose to call *Characium anophelesi* sp. nov. with the following diagnosis:

Cells pear-shaped, squat or elongated, broadly rounded at the top and narrowed gradually below into a rounded base, attached to the substratum by a thin round pad of mucilage; contents dividing into 2, 4 or 8 parts which escape by a clean rupture at the top; empty mother wall urn-shaped; small colonies sometimes formed; dimensions of the fully grown cells $41 \times 28 \mu$, $48 \times 22 \mu$, $48 \times 30 \mu$.

Hab. Growing densely on living larvae of several species of *Anopheles* mosquitoes in ponds in Sonarpur, Lower Bengal, India.

This species shows some resemblance to *C. saccatum* Filarszky which the author records as growing on *Branchypus stagnalis* Schaeff., but in *C. saccatum* the basal attaching mucilage is of a different form; in some cells Filarszky (cf. (2), Fig. 2, C, D) shows it disc-shaped, in others pocket-shaped with the lower end of the cell embedded in it ((2), Fig. 2, A, B). *C. anophelesi*, on the other hand, is attached by a very minute thin mucilage pad. In *C. saccatum* the cell opens by a vertical rupture ((3), Fig. 10), while in the present species a part of the apical wall of the cell breaks down to form a clear-cut aperture. The cells of *C. anophelesi* are far more variable in form and broader in proportion to their length than those of *C. saccatum*.

C. anophelesi also shows some resemblance to *C. Sieboldii* Braun, but the cells of this latter species commonly have a pointed apex, especially when young ((1), Tab. III, A, Figs. 1-17).

In conclusion the authors have much pleasure in thanking Prof. F. E. Fritsch for his kind help.

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EXPLANATION OF PLATE III

Characium anophelesi sp. nov. 1, a few cells on the body of a larva; the contents of some of the cells have divided into two (\times about 90); 2, dense growth of the alga on the abdominal segments of the larva (\times about 60); 3, a portion of the head of a larva enlarged to show the algal growth; some of the young *Characium* plants are growing on the empty parent-cells (\times about 130); 4, mature cells whose contents are beginning to divide (\times about 350); 5, head of a larva with a dense growth of *C. anophelesi* (\times 60).

A NOTE ON A WHITE FORM OF *PYRONEMA CONFLUENS*

BY W. J. BEAN AND F. T. BROOKS

Botany School, Cambridge

As *Pyronema confluens* is a fungus commonly used for class study it seems worth while to record the development of a white form of it in this laboratory. For several years a single isolation of this fungus has been kept in stock culture in tubes of Dox's agar, and has been used for the demonstration of the sexual organs after the manner of Claussen (1), although Dox's agar (without sugar in the outer dish) has been used instead of Claussen's medium.

In October, 1930, one of the plate cultures established by transferring sterile mycelium from a stock culture gave rise only to pure white apothecia, whereas other cultures produced the usual pink ascocarps. Since then the white form of this fungus has been sub-cultured repeatedly (both from mycelium and from spores) and has remained constantly white. There seems no doubt, therefore, that an albino form of *Pyronema confluens* has been permanently established.

In view of Barnes' work (2,3) on induced variations in fungi under the influence of high temperatures it was thought that possibly the needle had been overheated at the time of transferring the mycelium which developed into the white form. Repeated experiments, however, involving the use of an overheated needle, have failed to result again in the production of the white form of this fungus.

This permanently changed form of *Pyronema confluens* seems to be most comparable with the form of *Botrytis cinerea* having white sclerotia described by Brierley (4).

The only difference detectable between the white and pink types of *Pyronema confluens* concerns the pigment: the sexual organs and spores are identical in form and size.

Robinson (5) states that the pink pigment only appears in relation to reproductive activity. In our experience this is not true, for if the cultures are placed in sunlight the vegetative mycelium of the normal form frequently becomes bright pink. Under the same conditions the vegetative mycelium of the white form remains white.

A Note on a White Form of Pyronema confluens 71

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REVIEW

An Introduction to Plant Physiology. By W. O. JAMES, B.Sc., Ph.D., D.Phil. Pp. viii + 259, with 74 figures in the text (for readers of senior school or junior university status). Oxford University Press, 1931. Price 7s. 6d.

This book will be welcomed by teachers of botany throughout the country, for there is the severest need for a good elementary text-book of plant physiology in schools, a need which has been long felt. Nor will the welcome be misplaced, for Dr James gives a well-balanced presentation of the main features of the subject in a space of about 250 pages, and includes full and clear instructions for carrying out a series of 71 experiments suitable for use in school laboratories. This latter feature will, in our opinion, prove the most valuable part of the book. A large number of the experiments are not now in common use and yet might clearly be so; they have moreover the obvious virtues of methods tried out in laboratory classes by the writer himself. A table of reagents and methods of preparing them shows the care devoted to the experimental aspect of the book; this is a right emphasis and one which the author seems peculiarly well qualified to express.

Through being first in a field so long empty, the book will naturally be exposed to criticism from many angles by physiologists who dislike the author's presentation of one or other of the various aspects of plant physiology which have developed so rapidly in recent years. It will be unlikely to satisfy all plant physiologists because the subject is so wide that few if any individuals can now claim to be critically in touch with the whole of it.

The reviewer, certainly, is particularly dissatisfied with the presentation of the problems of water-uptake and the transpiration-stream. The discussion on factors affecting the passage of water into and out of cells is extremely short, the influence of organisation into tissue systems is almost neglected, and negative turgor pressures (i.e. outward wall pressures) are not even mentioned. It almost naturally follows that the exposition of water intake by roots should be rather disjointed and ineffective.

We feel that it would have been better to omit altogether the reference to that bane of botanical examiners in the "School Certificate" and similar examinations, the potometer built on the lines of a water-thermometer. It might be mentioned that *Mimosa pudica* is sensitive to touch as well as to utting or burning, and that *Lemna trisulca* does not float on the surface of

ponds. These are small points but they illustrate the wide variety of pitfalls awaiting the writer of the simple physiological text-book. It is of course impossible, in such a book as this, to present a highly critical account of progress in any field in which advance is being made; some fields are perhaps better neglected altogether and in some a rather arbitrary choice of material and attitude is advisable. In these difficult paths we should not always follow quite the same lines as Dr James, but his book is a practical solution of a real problem and must be treated as such. We would question the value of the discussion on diurnal changes in sugar content of leaves, since so little conclusion is possible from the results; and we doubt the advisability of discussing photosynthesis in the guard cells as a factor involved in the mechanism of stomatal movement, since it may so readily be confused with the basic mechanism of the sugar-starch balance. More serious objection may be taken to a misleading statement on p. 170 in the middle of a very good section on xerophytes. "Succulents contain large quantities of mucilages...and as a consequence succulent plants do not lose the water they have absorbed as easily as normal plants, or *mesophytes* lose theirs. The transpiration rate of an *Opuntia*, a typical succulent, was found to be about one-thirtieth of that of a normal mesophyte." The implication is that the presence of mucilage in *Opuntia* is solely responsible for its lower rate of transpiration. When it is recalled how many intervening factors, such as the size, number, opening and structure of the stomata, the internal air-space system, the conducting system, the root absorption area and distribution, and many more, may affect transpiration rates, and when also the units of surface area or of weight are not quoted, it will be evident that there is no sufficient basis, certainly no explained basis, for the implication made. Such examples of uncritical statement are however rare.

Broadly speaking the work is fundamentally sound and will prove of great service in the teaching of Botany, because it really does represent the first authoritative interpretation of modern plant physiology into terms simple enough for school use. We can hardly be too grateful for a book which gives a scientific account of the mechanism of stomatal movement in place of the old teleological comments, which clearly separates the starch-sugar hydrolysis and condensation system from photosynthesis, and which gives a simple picture of ionic absorption by roots and of some of the simpler aspects of plant respiration.

The language is generally simple and the knowledge of chemistry and physics expected not more than that assumed by other contemporary text-books for students of the same status. The text-figures are admirable and harmonise so very well with the clean and clear type that the book is really a very beautiful piece of printing.

H. G.

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ANATOMICAL VARIATION IN THE WOOD OF SOME DICOTYLEDONOUS TREES

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(With 11 figures in the text)

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INTRODUCTION

FOR nearly a century the scientific study of the structure of wood has engaged the attention of foresters and many workers have been perplexed by the variability in size and distribution of the elements. The subject has been approached from several view points: those interested in the identification of timbers have been concerned with variation between species, in the arrangement of the tissues and to a lesser extent in the dimensions of the cells; while those interested in the qualities or properties of timber have been concerned more with variations within the species or even within the individual tree, and more with the relative proportions of the different tissues and the size of cells than with their arrangement. Failure to realise the degree of variation within a species has resulted in the publication of descriptions based on insufficient material, which later

have been found to be inapplicable to much of the material subsequently collected.

The study of specific characters has so far received but slight attention. Saupe(20), as early as 1872, investigated the anatomical structure of the wood of the Leguminosae with a view to determining how far the relationship between closely allied genera can be demonstrated in the wood, and what particular features are most reliable for this purpose, and his conclusions testify to the complexity of the problem. More recently Moll and Janssonius(16) and other investigators have worked along similar lines, but much remains to be done before the identification of timbers can be considered to have been placed on a satisfactory basis. It is hoped that the present investigation, though not primarily designed to that end, may throw some light on this aspect by pointing to those features which show the least variation within the species, and which in consequence are likely to afford the most reliable distinctions.

The study of variation in size and distribution of the elements owes its inception to the work of Sanio(19), whose paper on the Scots pine was published in 1872. Sanio's work stimulated interest in the anatomical structure of wood and similar investigations of other important timber trees followed. In this connection the results of the investigations of Hartig(9, 10, 11), Stauffer(23) and Eichhorn(7), which appeared between 1885 and 1895, are noteworthy. Since the publication of these results, several other species have been studied both in this country and in America. Although some definite conclusions can be drawn as to the behaviour of cells in relation to their position in the tree, at the same time on certain points existing data is conflicting. For example Sanio(19) found that at a given height in the tree tracheid length increased outwards for a number of years, and then remained constant to the outside. Later investigations on other species have confirmed this initial increase outwards and shown it to be typical of all cell dimensions, but have failed to support the theory that cells eventually attain a constant size. For instance, an actual decrease to the outside, after the maximum has been attained, was observed in the beech by Hartig and Weber(9), in *Pinus palustris* Mill. by Shepard and Bailey(22), and in Douglas fir by Lee and Smith(15), although in the latter species the results were not confirmed by Gerry(8).

Observations regarding variation in the dimensions of cells of the same growth ring at different heights in the tree are also conflicting. Chalk(1) and several others have observed that the length of tracheids

in Gymnosperms increases upwards to a certain height in the tree and then decreases, but there is no such general agreement in the behaviour of fibres in the Angiosperms. A progressive decrease in the length of fibres from the base upwards was observed in beech by Hartig and Weber (9), in *Quercus robur* L. by Hartig (10, 11), and in birch by Stauffer (23), but in the American oaks Eichhorn (7) observed an increase up to a height of 16 feet, and then a decrease to the top of the tree, while in the shagbark hickory (*Carya alba* Nutt.) Prichard and Bailey (18) found that although the length of the fibres decreased from the base upwards, the vessel segments behaved differently and increased to a certain height and then decreased. Finally variation in specific gravity within a tree has been examined. Stauffer (23) observed, for example, that in the birch specific gravity increased from the centre outwards, while in the beech, another diffuse-porous wood, Hartig (9) found a progressive decrease from the centre outwards in most of the trees examined. In the oaks, Hartig and Eichhorn (10, 11 and 7) found that specific gravity usually decreased from the centre outwards, but in some trees there was an initial increase and then a decrease to the outside. One tree of beech behaved in a similar manner, the specific gravity at first increasing but finally decreasing outwards. It will be seen that as with variations in cell size the observed variations in specific gravity differ not only in different species but also within a species.

AIMS OF THE INVESTIGATION

(1) *Variation in cell size*

Variation in cell dimensions in relation to position in the tree was investigated with a view to solving two problems: (a) the course of variation outwards and upwards, and (b) the extent to which such variation is independent of species and external conditions. From a study of the existing literature it appeared that there were differences in the behaviour of different species and of individual trees and the question arose as to whether these differences were real or whether they could be accounted for by the inadequate methods then applied. The latter point raised the question of the degree of accuracy in measurement necessary to obtain comparable figures. This will be discussed more fully in the section dealing with methods.

(2) *Variation in the proportions of different tissues*

The aim here was the determination of the factors affecting the proportion of the different kinds of tissue in any sample.

(3) *Variation in specific gravity*

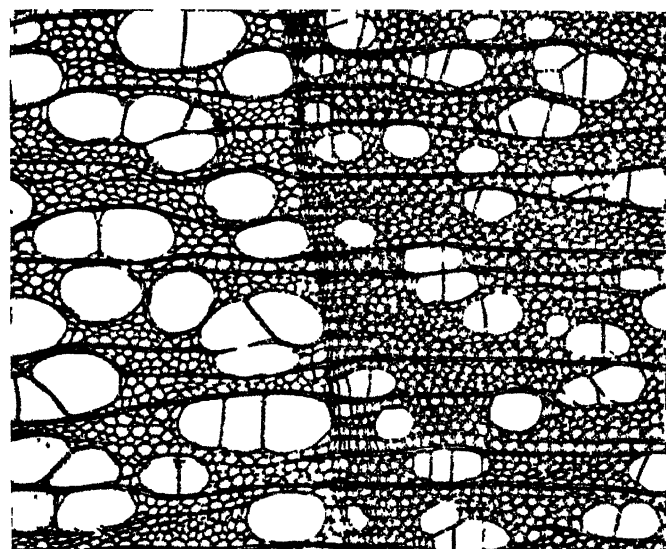
Specific gravity was investigated primarily for the purpose of deciding whether the changes outwards at any one height can be ascribed to inherent causes or whether they are governed mainly by external conditions. Here again differences which have been recorded in the behaviour of different species and of individual trees might be explained by defects in the methods used. The existing confusion therefore may be partly ascribed to faulty methods, but it still remains to be seen whether variation in specific gravity can be satisfactorily explained. It will be shown that in most trees specific gravity is lowest at the centre, increases outwards for a number of years and finally decreases, and that this sequence is not inconsistent with the effect of changing external conditions during the life of a normal forest-grown tree.

(4) *Methods*

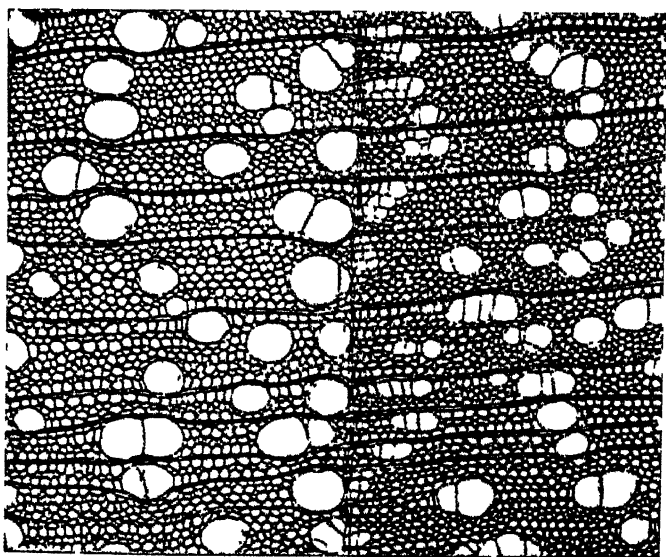
The first consideration was to determine the number of observations, for each particular type of measurement, that are necessary to obtain mean values of sufficient accuracy for purposes of comparison. Incidentally it was thought that the failure to make sufficient observations accounted for many of the discrepancies in the results of earlier workers. Other aspects to which attention was given were the possible effect of different methods of sampling and measuring.

The ultimate goal of such an investigation as this is the interpretation of the factors affecting the quality or strength of timber. The material and time at the disposal of the writer have made it impossible, for the present, to follow this investigation to its logical conclusion, but, until variation itself has been studied thoroughly, its significance as affecting the strength of timber is not likely to be fully understood. When it is possible to predict variation by knowledge of the factors causing it, it might then be possible to determine its significance.

The time involved in making large numbers of measurements has made it impossible to examine as many trees as the writer would have wished. It was decided that the object in mind could best be attained by examining trees from as wide a range as possible, and by the selection of several species in preference to restricting the investigation to a larger number of trees of a single species from one stand.



(b) 71st ring from the pith.
Poplar—*Populus serotina* Hartig. Tree P/I ($\times 75$.)



(a) 7th ring from the pith.

Fig. 1. Transverse sections showing variation in size of vessels at different distances from the pith.

MATERIAL

The material used in this investigation included seven trees of birch (four *Betula pubescens* Ehrh., and three *B. alba* L.), five of alder (*Alnus glutinosa* Gaertn.), two of beech (*Fagus sylvatica* L.), two of poplar (one *Populus canescens* Sm. and one probably *P. serotina* Hartig) and one each of sycamore (*Acer pseudoplatanus* L.) and Rhodesian teak (*Baikiaea plurijuga* Harms.). One or more transverse discs were cut from each tree, except in the case of beech tree F/I where small samples at different heights only were available; details are given in Table I.

TABLE I

Tree	Species	Locality	Height of discs or material in tree
A/I	<i>Alnus glutinosa</i> Gaertn.	Bagley Wood, nr Oxford	Base, 5' 6", 10', 14' 9" and 22' 6"
A/II	"	Owston Wood, nr Oakham, Rutland	Base and top of branch-free bole
A/III	"	"	Base, 7' 6", 18' 9", 26' 3" and 37' 6"
A/IV	"	"	Base, 5' 7", 14', 22' 5" and 30'
A/V	"	"	Base, 6', 15', 21' and 30'
B/I	<i>Betula pubescens</i> Ehrh.	University Parks, Oxford	Breast height
B/II	"	"	"
B/III	<i>Betula alba</i> L.	North of Scotland	Three discs at 3' 4" intervals
B/IV	"	Bagley Wood, nr Oxford	Base, 5', 11' 6", 17' and 25'
B/V	<i>Betula pubescens</i> Ehrh.	Owston Wood, nr Oakham, Rutland	Base, 7', 17', 24' and 34'
B/VI	"	"	Base, 6' 6", 16', 22' 6" and 32'
B/VII	<i>Betula alba</i> L.	"	Base, 6', 15', 21' and 30'
F/I	<i>Fagus sylvatica</i> L.	Cotswolds	Samples from the outside at base, 5', 10', 15', 25', 30' and 35'
F/II	"	Chilterns	Disc at 6'
P/I	<i>Populus serotina</i> Hartig	Bagley Wood, nr Oxford	Base
P/II	<i>Populus canescens</i> Sm.	University Parks	Base, 5' 6", 11' and 23' 6"
S/I	<i>Acer pseudoplatanus</i> L.	Bagley Wood, nr Oxford	Base, 5', 10', 15', 24' and 32'
T/I	<i>Baikiaea plurijuga</i> Harms.	Siburu Forest, N. Rhodesia	Base

Identification of the specimens was carried out by Dr Burt Davy and Mr Hoyle at the Imperial Forestry Institute, from herbarium material, except in the case of birch, tree B/III, and Rhodesian teak, tree T/I, for which no herbarium material was available.

METHODS

Determination of specific gravity

"Green" or saturated volume and oven-dry weight were used in the calculation of specific gravity¹. Blocks were cut out from one radius only of each disc, but always from the same side of the tree where more than one disc was available. The saturated volumes were obtained by placing the blocks in a beaker of water and exhausting the air by means of a vacuum pump. When waterlogged, the blocks were left under water at normal atmospheric pressure for one week; after which the volumes were determined. To test the accuracy of various methods a series of blocks were cut from the disc of *Baikiaea* and their volumes determined in three different ways.

(a) By measurement of length, breadth and height of blocks with callipers reading to 0.01 mm., the average of nine measurements being taken for each dimension and the mean of two sets of measurements for the volume.

(b) By immersion in a balanced beaker of water, the block being held in position by a needle; the amount of water displaced being indicated by the weights added to the pan to restore balance.

(c) By the difference between weight in water and weight in air, the block being suspended from the balance by a fine thread.

The different methods were found to give closely similar figures for the saturated volume; the extreme variation in volume for any one sample did not exceed 1.25 per cent. Table II gives the results

TABLE II
*Variation in saturated volume due to differences in
methods of determination*

	Method (a)		Method (b)	Method (c)	Mean
	(1)	(2)			
Block II	1.0000	1.0036	1.0036	1.0000	1.0001
Block V a	1.0000	1.0000	0.9886	0.9886	0.9943
Block VII a	1.0000	—	0.9856	0.9856	0.9904

¹ The ratio $\frac{\text{Oven-dry wt.}}{\text{Sat. vol.}}$ is not a true specific gravity, as the comparison is between mass in one state and weight in another. The term specific gravity has, however, been retained for my own figures throughout this investigation for want of a better term.

for the three samples showing the most variation; the volume obtained by method (a) has been expressed as unity and the volumes obtained by the other methods made proportional.

The different figures obtained for the saturated volume calculated by the three methods have only a small effect on the value calculated for specific gravity. In no case did the value of the calculated specific gravity vary by more than 0.0118.

TABLE III
*Effect of variation in saturated volume determinations
on specific gravity*

	Method (a)		Method (b)	Method (c)	Mean
	(1)	(2)			
Block II	1.0000	0.9981	0.9992	1.0000	0.9995
Block V a	1.0000	1.0000	1.0205	1.0205	1.0076
Block VII a	1.0000	—	1.0147	1.0147	1.0097

Table III shows the variation in specific gravity due to the variations in saturated volume determinations. The specific gravity calculated from the saturated volume obtained by method (a) (1) has been expressed as unity and the specific gravities obtained by using the saturated volume obtained by the other methods have been made proportional.

From these tables it will be seen that the amount of variation due to the different methods is unimportant and that, provided the same method was used throughout, the results should be comparable. Method (b) was selected partly on the grounds of rapidity and partly to permit, when necessary, of the use of irregularly shaped blocks.

After the volumes had been determined the blocks were allowed to dry out at ordinary laboratory temperatures for one week, and were then placed in an electric oven maintained at 60° C. for four days; they were then transferred to an oven at 104° C., until no further significant loss in weight occurred between daily weighings, and the oven-dry weight was then determined.

Number of observations

The measurement of the diameter of vessels and the length of fibres presented greater difficulties than the determination of specific gravity, as these elements exhibit very considerable range in dimensions in any one sample. The practice of earlier workers of limiting their observations to 30–50 measurements is inadequate. The number of measurements should be large enough to constitute a representative sample.

In the description of a wood for purposes of identification it is desirable to know the range in cell dimensions; but the arithmetic mean is the more useful criterion when comparing adjacent samples from the same tree, as the ranges are very similar and overlap. As it is practicable to measure only a limited number of cells from any one sample, and all methods of measurement are limited in accuracy, it is necessary to determine what significance can be placed on the data obtained by sampling. A simple example will make the position clear.

The fibre length at different heights in the tree was studied in the case of an alder, samples being taken to include the last three annual rings only, and the following figures (each being the mean of 300 measurements) were obtained:

Height in tree ft.	Length of fibres in μ	Standard error
Base	1144	± 6.2
6	1170	± 6.8
15	1186	± 6.0
21	1130	± 6.1
30	1052	± 5.6

Are these figures sufficiently accurate to justify the conclusion that in this tree fibre length increased upwards to a height of 15 ft. or more and then decreased?

The question of the significance of data has recurred throughout this investigation, and unless the answer can be given in the affirmative no definite conclusions can be drawn. Steven (24) states that "there are four values, or to use Fisher's term 'statistics,' which are constantly employed in statistical methods relating to experimental work. These 'statistics' are (a) the mean, (b) the standard deviation of the observations on which the mean is based, (c) the standard error of the mean to avoid confusion with (b), and (d) the standard error of the mean of differences. It is important to note that none of these statistics represents an absolute value when applied to ordinary experimental data, *all are estimates only of the true values.*" The degree of accuracy required depends on the difference between the means of samples to be compared, and if this difference is small the number of measurements necessary (to ensure a narrow range in the mean) must be large.

The mean is obtained by summing the individual observations and dividing by n (number of observations). The standard error of the mean was calculated in two ways depending on the number of observations. For a large number of observations, e.g. 100 or more,

the method described by Thurstone⁽²⁵⁾, using class intervals and an arbitrary origin was employed, the formula being

$$E = \frac{\sqrt{\frac{\sum (fd^2)}{n} - c^2}}{\sqrt{n}} \times \text{class intervals},$$

where f = frequencies in each class interval, d = deviation from arbitrary origin, $c = \frac{\sum (fd)}{n}$, and n = the number of observations (see Thurstone, Chapter xv). For a smaller number of observations (e.g. 50), the formula

$$E = \frac{\sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}}{\sqrt{n}}$$

was used. That is, each value is subtracted in turn from the mean, the squares of the differences are summed and divided by $n - 1$ and the square root of the quotient is divided by the square root of n .

The standard error of the mean forms a measure of the normal distribution of the mean, and from it the probability that the true mean of the "population" lies within a certain range on either side of the calculated mean can be determined. Discussing the question of the practical application of the standard error of the mean, Steven⁽²⁴⁾, p. 172 says: "It can be shown that for a large sample, drawn from a normally distributed population, the chances are about 3 to 1 that the mean lies within the range measured by the standard error; the chances are nearly 22 to 1 that the mean lies within twice the standard error, and 370 to 1 that it lies within three times the standard error."

By the use of statistical methods, then, the probable degree of reliability of differences between figures obtained for different samples may be determined¹.

A probability of 22 to 1 has usually been accepted as sufficient for most forms of experimental work, hence if the difference between

¹ It should be pointed out, however, that the above statement is only true when the sampling is representative. If the sampling were not representative it is possible that the difference between two estimates might appear significant when in actual fact no significant difference existed. In other words the statistical treatment of figures does not correct bad sampling, nor does it eliminate the personal equation in the selection of samples. A further limitation to the application of statistical methods in biological work is the accuracy of measurement. It may be impossible to prove the significance of differences by statistical methods, not because a significant difference does not exist, but because the method of measurement is not sufficiently accurate.

the means of two samples is twice the standard error of the difference, then the difference is significant. The formula for determining the standard error of the difference is

$$E_d = \sqrt{(E_x^2 + E_y^2)},$$

where E_d = standard error of the difference and E_x and E_y the standard errors respectively of the means x and y .

For example it is found that the mean fibre length at a height x in the tree is 1144μ , while at y it is 1186μ ; neither of these figures is an absolute value as both are means of samples; is the difference significant? The standard errors of the means have been calculated and found to be ± 6.2 and ± 6.0 respectively. Then

$$E_d = \sqrt{(6.2)^2 + (6.0)^2} = \pm 8.6.$$

The difference between the means x and y is 42, which is nearly five times the standard error of the difference and is therefore significant.

If a large number of random observations be made of any population, the frequencies with which similar individuals occur are found to lie on a "distribution curve," and for a given number of observations, the standard deviation S may be calculated. Now if the number of observations be doubled the proportions in which the similar individuals are present is unaltered, and there is no change in the standard deviation. The standard error of the mean, however, will be reduced inversely as the square root of the number of observations, since for the standard deviation of the mean $E = \frac{S}{\sqrt{n}}$.

The effect of increasing the number of observations will be readily seen when E , in the equation $E = \frac{K}{\sqrt{n}}$, is plotted against the number of observations. (The standard deviation not being affected by the number of observations is treated as a constant K , though this of course is not necessarily strictly true.)

From the graph (Fig. 2) it will be seen that the standard error of the mean is reduced by one-half if the number of observations is increased from 100 to 400, but to reduce the standard error again by one-half a further 1200 (1600 in all) observations are necessary. The time involved in measuring a large number of cells is considerable, and since it was found that the standard error based on 300 observations was sufficiently small in most cases to show the significance of differences between the means of different samples,

this number of observations was adopted for the purposes of this investigation.

Methods of measurement

Fibre length. Macerated material mounted in glycerine jelly to which a little gentian violet had been added was used for the measurement of fibre length. Small tangential chips of wood were macerated in Schultz's maceration fluid. Very small quantities of the macerated

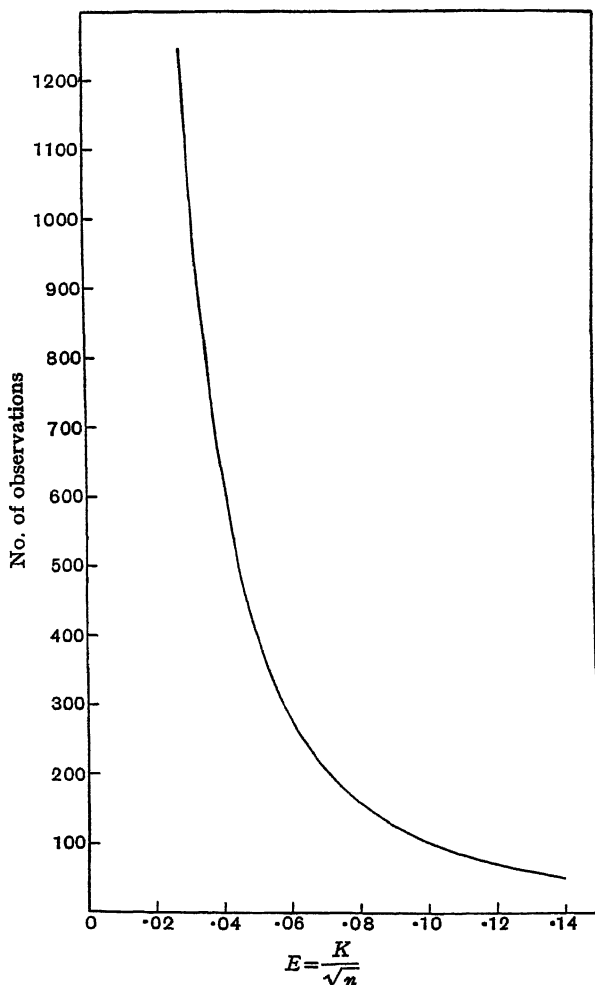


Fig. 2. Graph showing the effect of the number of observations on the standard error when the standard deviation is constant.

material were mounted on each slide to ensure as far as possible the measurement of every unbroken fibre, thereby avoiding selection in the measurement of isolated cells. The advantage of tangential over radial chips has been mentioned by Chalk(1); radial chips give a preponderance of cells from the same cambial initial rather than a representative selection covering the range in any one sample. It was difficult to eliminate errors in measurement entirely when using macerated material, as the largest cells were more likely to be damaged in mounting, while unusually short cells tended to be excluded in measuring, as the low magnification at which measurements were made rendered it difficult to decide in some cases whether a cell was broken or not.

Measurements were made by means of a Leitz projection apparatus giving, on a screen, a direct image magnified 40 times. A scale with divisions representing 50μ was constructed with the aid of a stage micrometer.

Vessel diameter. Thin transverse sections were cut from blocks adjacent to those selected for determination of specific gravity. The sections were mounted in glycerine jelly to which a little gentian violet had been added, or were stained in Haidenhain's haematoxylin and safranin and mounted in Canada balsam.

Measurement of vessel diameter was facilitated by means of a simple projection apparatus, as constructed by Clarke(6). A scale with divisions representing 5μ was used, and the radial and tangential diameters of the lumina of solitary vessels were measured. It was found that sets of 100 measurements grouped in 5μ classes gave similar frequency curves for any one sample. The difference in the arithmetic mean of any two sets of 100 measurements was rarely more than 3 per cent. The high magnification at which measurements were made reduced the error of measurement to a minimum, while the measuring of each solitary vessel in the section in sequence eliminated errors of selective or "chance" measurement.

Vessel area. Three methods of determining vessel area were investigated:

(a) Photomicrographic method; the area being calculated on the basis of the ratio by weight of vessels cut out from a negative print to the weight of the whole print.

(b) By means of a Shand's recording micrometer.

(c) By calculating the approximate number of vessels and vessel groups in a given area of cross-section, and multiplying this number by the average area of the vessels. The average area was calculated

from the measurement of the image obtained by a Leitz projection apparatus using a magnification of 165 times. The mean radii of solitary vessels were grouped in 5μ classes, the area of each class being calculated from the formula $n(\pi r^2)$, where n is the frequency and r the mean radius of the class intervals. The vessel groups were treated as ellipses, the area being calculated from the formula

$$n \times \frac{(a \times b)}{4} \times \pi,$$

where n is the number of vessel groups and a and b the means respectively of all the tangential and radial diameters.

In the determinations of vessel area three factors have to be considered, (1) limitations of the methods used, (2) the necessity of examining a sufficiently large field, owing to irregularities in vessel distribution in one annual ring and the considerable range from ring to ring, and (3) time necessary to make the determination.

The photomicrographic method, which at first sight appeared to offer a satisfactory solution, was rejected, as too high a magnification was necessary to permit a sufficient accuracy in cutting out the vessels. To use this method would have involved either restricting the determinations to a very small area, thereby introducing errors due to irregularities in vessel distribution, or alternatively making several photomicrographs to cover a much larger area of the section, but this was not practicable as the time involved in cutting out the vessels is considerable. The degree of accuracy in cutting out was tested on a section of birch at a magnification of 75 times, and was found to be about ± 5 per cent. When the errors due to irregularities in vessel distribution are also considered it will be seen that unless a comparatively large field is examined the accuracy of this method is insufficient to justify its use.

The second method investigated was the use of a Shand's recording micrometer which Clarke(5) had found satisfactory in elm for measuring the proportion of fibres, fibre wall substance, rays and summer wood in any sample. The method was tested by taking thirty-two readings, at equal distances apart, diagonally across a transverse section of birch and repeating the determinations with thirty readings on the same section, the second series alternating with the first. The proportion by area of vessels was found to be 13.08 and 13.43 per cent. respectively for the first and second series of measurements. The range between the individual readings was considerable, being for the first series from 7.90 to 18.30 per cent.

and for the second from 8.20 to 18.02 per cent. Obviously the greater the number of readings the more closely does the value obtained approach the true value, hence in this case a better value for vessel area would be the mean of the sixty-two readings which was found to be 13.25 per cent. The standard error of this mean was calculated and found to be ± 0.31 . We may then say with reasonable certainty that the true mean lies somewhere within the range measured by twice this standard error.

For this method to be of practical application it is necessary to determine what amount of difference between two means must exist for it to be possible to prove its significance. This difference may be expressed approximately as a percentage, using the formula

$$\sqrt{(E_x^2) + (E_y^2)}$$

to obtain the standard error of the difference. Then the percentage difference for a probability of 22 to 1 is obtained as follows:

$$\frac{2 \times \text{standard error of differences}}{\text{calculated mean of sample}} \times 100.$$

In the same species the value of E_x or E_y will depend primarily on the number of observations. If in the example referred to above the calculated standard error of the mean for sixty-two readings is substituted for both E_x and E_y the formula then becomes

$$\frac{2 \times \sqrt{2 (0.31^2)}}{13.25} \times 100,$$

giving a value of 7.4 per cent. A difference between two means of this order or less may be expected with sixty-two readings, any

TABLE IV

Decrease in accuracy in determination of vessel area resulting from decrease in number of readings

Readings		Mean vessel area (%)	Standard error of mean	% difference necessary to prove significance
62, Nos.	1,, 62	13.25	± 0.31	7.4
32, "	1,, 32	13.08	± 0.47	10.0
30, "	33,, 62	13.43	± 0.45	
16, "	1, 3, 5, ..., 31	12.52	± 0.69	14.0
16, "	2, 4, 6, ..., 32	13.64	± 0.63	
11, "	1, 4, 7, ..., 30	13.37	± 0.73	16.0
11, "	2, 5, 8, ..., 31	12.71	± 0.73	
8, "	1, 5, 9, ..., 29	11.80	± 1.15	21.0
8, "	2, 6, 10, ..., 30	12.50	± 0.88	
8, "	3, 7, 11, ..., 31	13.24	± 0.84	
8, "	4, 8, 12, ..., 32	14.77	± 1.02	

difference exceeding this value would be significant. The effect of reducing the number of readings is of course to increase the amount of difference which must exist for its significance to be shown statistically. This was studied for the series of sixty-two readings referred to above, and the results are given in Table IV.

From the table it will be seen that a large number of readings is necessary to show the significance of small differences. The method was, however, applied to determine vessel area per cent. in disc 1 of birch tree B/I, the determinations being based on fifty readings for each section examined, and the results are given in Table V.

TABLE V

Birch tree B/I. Disc 1. Vessel area per cent. at different ages, calculated by method (b). For description see text

Age of ring in years	Vessel area (%)	Standard error	Difference of means
			Standard error of difference*
5	14.9	±0.35	4.8
10	12.2	±0.44	1.4
15	11.5	±0.29	0.7
20	11.2	±0.33	2.6
25	12.6	±0.41	

* The difference between two means is significant when

$$\frac{\text{Difference}}{\text{Standard error of difference}} > 2.$$

Table V shows that satisfactory results may be obtained with a large number of readings but the amount of time involved in making so many readings is a drawback to the general use of this method.

The third method was therefore tested, firstly to determine how closely the calculated area approached the true area, and secondly what degree of accuracy in measurement could be obtained with a magnification of 165 times. The first point was investigated by drawing under the high power of the microscope twenty-two consecutive solitary vessels, and fifteen vessel groups. The approximately true areas were obtained with a planimeter; the solitary vessels amounting to 86.34 and the vessel groups to 149.33 sq. in., while the calculated areas were respectively 91.36 and 152.15 sq. in., giving a positive error for the total of 3 per cent. This error was considered sufficiently small to be unimportant, and being constant in direction would not affect the figures obtained from successive samples from the same tree. The second point was examined by

carrying out the measurement of vessels on 5.59 sq. mm. of one annual ring, and repeating the readings for the identical portion of the ring, the figures obtained being as follows:

	Vessel area	Ring area	Percentage of vessels by area
1st series	1.365	5.59	24.4
2nd series	1.348	5.59	24.1

Thus the error of measurement is only about 1.25 per cent. In this method the area examined was considerably larger than that in either of the other two methods, while the time taken for the determinations was much less. For this reason it was decided to adopt method (c) for vessel area determinations. An additional advantage of this method is that in the case of all species, except those with aggregate rays, ray volume can be determined at the same time as vessel area.

RESULTS AND DISCUSSION

(1) *Variation in cell size*

It has been generally observed that striking changes in cell size occur between the pith and the bark, that is to say outwards, at a given height in the tree (Fig. 1). The changes in this plane were therefore investigated first. Details of the measurements of fibre length and vessel diameter are given in Tables VI–XIII. The figures in columns 2, 5 and 8 are each the arithmetic mean of 300 observations. The standard error of each mean, calculated by the method described on p. 10, is given in columns 3, 6 and 9. The significance of the difference between any two means is given in the column “measure of significance.” When the figure in this column is 2.0 or greater, the chances are 22 to 1, or more than 22 to 1, that the difference between the mean of this sample and the one below it is significant, but when less than 2.0 the chances are less than 22 to 1, and the differences cannot be regarded as significant (Tables VI–XIII).

The figures in the tables show that at a given height in the tree cell size increases outwards from the pith and is subject to irregular fluctuations. From the column “measure of significance” it can be seen that in most cases the differences are significant; and therefore the fluctuations are real. Fluctuations occurring in one dimension were found to coincide, in general, with fluctuations in the other dimensions, indicating that the changes in the different dimensions

measured were governed by a common factor or factors. That an increase in vessel diameter should be accompanied by an increase in fibre length was to be expected if one accepts the view that the elongation of the fibres is caused by the pressure exerted by the swelling vessels. The amount of increase in cell size from the pith to the outside varied in the different trees and in the different dimensions examined, but in all cases was considerable. In birch (tree B/I), for example, increase in fibre length amounted to slightly over 60 per cent., and in vessel diameter to over 100 per cent.

TABLE VI

Alder tree A/II, age 102 years. Mean fibre length and vessel diameter at different ages at the base

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1929	1147	± 7.6		58	± 0.56		79	± 0.80	
1913	1184	± 6.4	3.7	56	± 0.56	2.5	77	± 0.83	1.7
1898	1132	± 7.1	5.4	53	± 0.59	3.7	74	± 0.88	2.4
1884	1108	± 5.8	2.4	48	± 0.53	6.3	74	± 0.79	—
1875	1113	± 7.1	0.54	47	± 0.56	1.3	65	± 0.78	8.1
1865	1073	± 5.7	4.3	49	± 0.54	2.5	64	± 0.72	0.9
1854	1113	± 7.3	4.3	46	± 0.51	4.0	65	± 0.70	1.0
1844	1099	± 8.0	1.3	41	± 0.46	7.3	67	± 0.68	2.0
1834	1056	± 5.9	4.1	36	± 0.51	7.3	49	± 0.56	20.4

TABLE VII

Alder tree A/IV, age 93 years. Mean fibre length and vessel diameter at different ages at the base

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1930	1183	± 8.1		52	± 0.50		74	± 0.64	
1919	1242	± 8.6	4.8	57	± 0.54	6.8	78	± 0.71	4.2
1910	1182	± 6.8	5.6	55	± 0.54	2.6	77	± 0.69	1.0
1900	1179	± 6.9	—	54	± 0.54	1.3	74	± 0.73	3.0
1890	1118	± 6.4	6.4	51	± 0.50	4.1	74	± 0.73	3.0
1880	1211	± 7.0	9.8	52	± 0.50	1.4	77	± 0.73	—
1870	1140	± 7.2	7.1	51	± 0.55	1.3	71	± 0.70	6.0
1860	1173	± 8.1	3.1	49	± 0.54	2.6	75	± 0.93	5.2
1849	1034	± 5.3	14.4	47	± 0.48	2.8	71	± 0.66	3.5
1845	999	± 4.8	4.9	43	± 0.54	5.5	75	± 0.96	3.4

TABLE VIII

Birch tree B/I, age 27 years. Mean fibre length and vessel diameter at different ages at 4 ft. 3 in.

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. tan. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. rad. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1929	1363	± 8.6	—	68	± 0.64	—	91	± 0.84	7.6
1924	—	—	—	61	± 0.62	7.9	82	± 0.83	2.6
1919	1135	± 7.1	19.0	58	± 0.66	3.3	79	± 0.78	4.9
1914	946	± 5.6	21.1	51	± 0.46	8.7	74	± 0.67	16.9
1911	840	± 5.3	13.5	41	± 0.38	16.8	57	± 0.75	14.2
1908	—	—	—	32	± 0.43	15.6	44	± 0.52	—

TABLE IX

Birch tree B/IV, age 56 years. Mean fibre length and vessel diameter at different ages at the base

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. tan. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. rad. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1929	1328	± 7.9	—	61	± 0.89	—	94	± 1.4	9.6
1925	—	—	—	74	± 0.92	10.2	113	± 1.4	0.5
1919	1505	± 8.1	15.6	70	± 0.91	3.1	112	± 1.4	1.7
1909	1403	± 9.8	8.0	76	± 1.20	4.0	116	± 1.9	3.2
1899	1323	± 7.3	6.5	73	± 0.98	1.9	108	± 1.6	3.3
1890	1296	± 7.3	2.6	74	± 0.86	0.8	115	± 1.4	22.8
1881	1042	± 5.3	28.1	50	± 0.66	22.1	76	± 0.98	0.7
1878	—	—	—	50	± 0.64	—	77	± 0.95	—

TABLE X

Beech tree F/II, age 129 years. Mean fibre length and vessel diameter at different ages at 6 ft.

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. tan. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. rad. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1930	1096	± 6.2	—	51	± 0.64	—	62	± 0.80	6.6
1926	—	—	—	58	± 0.79	7.0	71	± 1.10	0.7
1921	1265	± 7.1	17.4	56	± 0.54	2.1	72	± 0.83	0.8
1910	1244	± 8.4	1.9	58	± 0.65	2.4	73	± 0.81	0.8
1902	1206	± 7.2	3.4	56	± 0.65	2.2	72	± 0.87	7.4
1893	1289	± 8.5	7.5	49	± 0.61	7.9	63	± 0.86	5.7
1882	1249	± 7.1	3.6	54	± 0.59	5.9	70	± 0.87	4.3
1871	1270	± 8.0	1.9	50	± 0.56	4.9	65	± 0.76	11.5
1855	1116	± 8.0	14.6	44	± 0.50	8.0	54	± 0.59	1.2
1840	1165	± 6.9	4.3	45	± 0.43	1.5	55	± 0.58	4.4
1832	1183	± 8.9	1.6	46	± 0.52	1.5	59	± 0.70	4.0
1821	1164	± 7.1	1.7	50	± 0.46	5.8	63	± 0.70	—
1806	1002	± 7.0	16.3	—	—	—	—	—	—

TABLE XI

Poplar tree P/I, age 102 years. Mean fibre length and vessel diameter at different ages at the base

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. tan. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. rad. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1928	1455	± 8.5	0.8	84	± 1.10	2.1	133	± 1.6	1.3
1918	1445	± 8.5	2.6	81	± 0.94	2.2	136	± 1.6	2.2
1908	1480	± 10.4	0.1	84	± 0.98	5.4	131	± 1.6	1.9
1898	1482	± 10.3	5.3	76	± 1.10	2.2	126	± 2.0	7.1
1889	1408	± 9.4	4.0	73	± 0.77	5.1	109	± 1.3	9.7
1879	1464	± 10.2	1.7	79	± 0.89	7.1	129	± 1.6	6.4
1870	1438	± 10.9	8.7	70	± 0.90	0.8	115	± 1.5	1.9
1859	1324	± 7.2	12.3	69	± 0.89	1.6	119	± 1.5	2.4
1853	1207	± 6.3	0.5	67	± 0.87	2.4	114	± 1.4	4.0
1846	1202	± 7.8	8.4	64	± 0.89	3.4	106	± 1.4	4.5
1838	1121	± 5.6	10.6	60	± 0.79	6.6	98	± 1.1	16.3
1835	1038	± 5.5		53	± 0.71		75	± 0.88	

TABLE XII

Sycamore tree S/I, age 56 years. Mean fibre length and vessel diameter at different ages at the base

Ring (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. tan. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. rad. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
1930	906	± 5.2	4.6	66	± 0.65	1.1	79	± 0.85	0.8
1920	875	± 4.3	1.7	65	± 0.60	1.1	78	± 0.85	2.5
1911	863	± 5.6	4.7	64	± 0.63	3.3	75	± 0.83	2.5
1900	828	± 4.9	6.0	61	± 0.67	2.1	72	± 0.86	0.9
1892	789	± 4.2	8.0	63	± 0.65	5.4	73	± 0.77	3.6
1884	—	—	—	58	± 0.65	6.1	67	± 0.80	3.9
1879	744	± 3.7		53	± 0.51		63	± 0.66	

TABLE XIII

Rhodesian teak tree T/I. Mean fibre length and vessel diameter at different distances from the pith at the base

Distance from pith (1)	Mean fibre length in μ (2)	Standard error (3)	Measure of signifi- cance (4)	Mean vessel diam. tan. in μ (5)	Standard error (6)	Measure of signifi- cance (7)	Mean vessel diam. rad. in μ (8)	Standard error (9)	Measure of signifi- cance (10)
12"	929	± 5.6	1.2	65	± 0.58		82	± 0.85	3.6
10 1/2"	919	± 6.1	0.8	70	± 0.69	5.5	87	± 1.10	0.5
9 1/2"	912	± 6.6	0.2	69	± 0.75	1.0	86	± 1.05	1.4
7 1/2"	910	± 6.1	0.9	69	± 0.68	—	88	± 1.01	11.1
6 1/2"	918	± 6.0	2.9	61	± 0.69	8.2	73	± 0.89	13.2
4 1/2"	894	± 5.7	0.1	50	± 0.58	12.2	58	± 0.70	4.3
3 1/2"	893	± 5.8	0.6	46	± 0.50	5.2	54	± 0.62	1.1
1 1/2"	897	± 4.5	7.7	45	± 0.46	1.5	55	± 0.69	8.0
1/2"	850	± 4.1		43	± 0.44	3.1	48	± 0.54	

It is not easy to determine from the tables either the duration of the period of increase in cell size or whether the rate of increase is greatest in early youth as has been stated by some workers. Graphs were therefore constructed, showing the variation of fibre length and vessel diameter in relation to age, with the addition of a curve of the periodic mean annual area increment. The figures

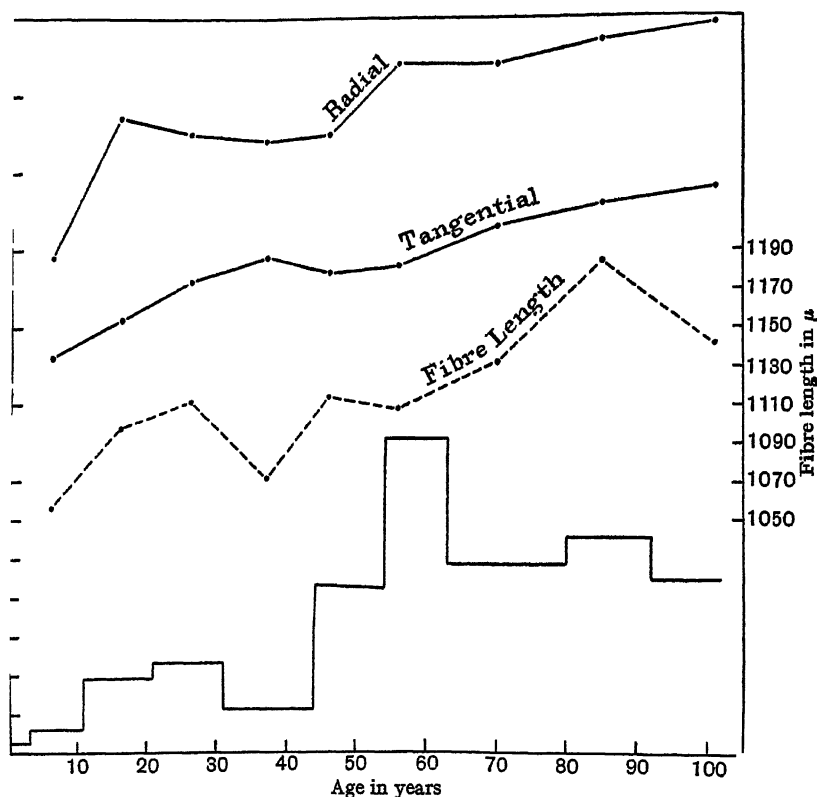


Fig. 3. Alder tree A/II. Fibre length, vessel diameter and periodic mean annual area increment at the base plotted against age.

(3 to 10) show that in most trees the period of most rapid increase in cell size was restricted to the first five to ten years, and that subsequently size continued to increase steadily. Further, if the decrease in the last ring which occurred in practically all trees is excepted, cell size was still increasing in the beech at 129 years of age, and in the poplar and alder at 102 years of age, and in the sycamore and birch at 56 years of age. The decrease in cell size in

the last ring was accompanied by a marked decrease both in the rate of increment and in the annual increment; and this suggested the possibility of a relationship between cell dimensions and either or both of these features. Further the fact that this decrease occurred simultaneously in several trees indicates that in this case meteorological conditions were the predominant factor.

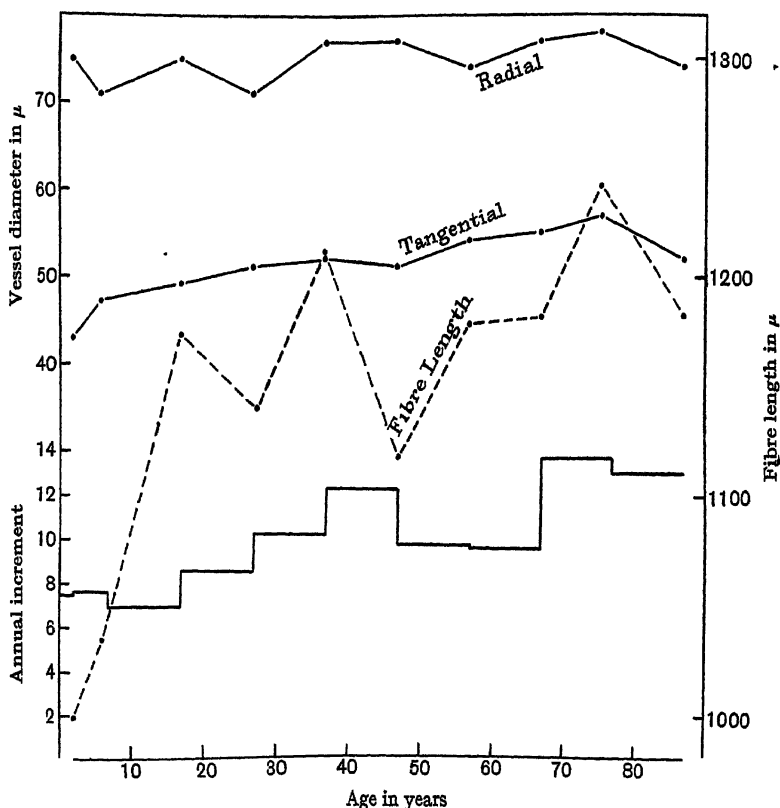


Fig. 4. Alder tree A/IV. Fibre length, vessel diameter and periodic mean annual area increment at the base plotted against age.

From the graphs it can be seen that the relation between cell dimensions and annual increment is similar throughout. Correlation coefficients (26) were therefore calculated, using the formula

$$r = \frac{\sum xy}{n \cdot \sigma_x \cdot \sigma_y}, \text{ error} = \pm \frac{\sqrt{1 - r^2}}{\sqrt{n}},$$

and the coefficients obtained are given in Table XIV below.

TABLE XIV

Correlation coefficients for cell dimensions and periodic mean annual area increment

Tree No.	Fibre length		Tan. vessel diam.		Rad. vessel diam.	
	Correlation	Standard error	Correlation	Standard error	Correlation	Standard error
Alder A/II	+·637	±·1946	+·585	±·2198	+·752	±·1450
„ A/IV	+·810	±·1089	+·853	±·0861	+·578	±·2106
Poplar P/I	+·925	±·0419	+·885	±·0628	+·895	±·0577
Birch B/IV	+·861	±·1060	+·693	±·1838	+·732	±·1640
Sycamore S/I	+·914	±·0674	+·791	±·1415	+·883	±·0832
Beech F/II	+·418	±·2392	+·589	±·1883	+·540	±·2113

In spite of the fact that the calculations were based on comparatively few figures, in no case more than twelve, the correlation coefficients show the existence of a close relationship between cell size and annual increment. Fibre length generally gave the highest coefficient, and the radial diameter of the vessels gave a higher coefficient than the tangential diameter. The high correlation between cell dimensions and annual increment would lead one to expect the increase in the increment to be greatest during the first five to ten years, since the greatest increase in cell size was in this period; that this is so can be seen in Figs. 3-9. Conversely, it might be argued that the initial rapid rise in cell size can be attributed to the rapid rise in increment which is usual during this period rather than to any inherent character of the tree but this appears improbable.

The correlation coefficients refer only to the first hundred years or less in the life of each tree, but from the closeness of the correlation one may perhaps assume that a similar relationship persists also in the later life of the tree. If this assumption is correct there is considerable justification for drawing conclusions as to the changes in cell size in this later period. The annual increment and therefore cell size is likely to be maintained to a considerable age, but when the tree has reached the stage of what is technically called over-mature, the increment falls and therefore a decline in cell size is to be expected. Evidence in support of this ultimate decline in cell size is found in Hartig's⁽⁹⁾ figures for cell length in the beech, and the fact that a decline in cell size has not been observed by many workers may be explained by the immaturity of their material.

The examination of variation in cell size in one annual ring at different heights in the tree was restricted to the determination of fibre length. Details of the figures obtained are given in Tables XV-XVIII.

TABLE XV

Alder tree A/II, ring 1930. Mean fibre length in one annual ring at different heights in the tree

Height in tree	Mean fibre length in μ	Standard error	Measure of significance
Base	1144	± 6.2	
6' 0"	1170	± 6.8	2.84
15' 0"	1186	± 6.0	1.71
21' 0"	1139	± 6.1	5.49
30' 0"	1052	± 5.6	10.50

TABLE XVI

Sycamore tree S/I, ring 1930. Mean fibre length in one annual ring at different heights in the tree

Height in tree	Mean fibre length in μ	Standard error	Measure of significance
Base	784	± 4.2	
5' 0"	892	± 5.0	16.55
10' 0"	817	± 5.6	10.00
15' 0"	819	± 5.0	0.27
24' 0"	809	± 4.5	1.49
32' 0"	735	± 4.3	11.90

TABLE XVII

Beech tree F/I, ring 1930. Mean fibre length in one annual ring at different heights in the tree

Height in tree	Mean fibre length in μ	Standard error	Measure of significance
Base	1307	± 12.0	
5' 0"	1365	± 11.6	3.48
10' 0"	1318	± 10.9	2.96
15' 0"	1320	± 10.8	0.13
20' 0"	1347	± 12.2	1.65
25' 0"	1235	± 10.4	6.97
30' 0"	1293	± 12.6	3.55
35' 0"	1263	± 11.9	1.73

TABLE XVIII

Poplar tree P/II, ring 1930. Mean fibre length in one annual ring at different heights in the tree

Height in tree	Mean fibre length in μ	Standard error	Measure of significance
Base	1178	± 7.3	
5' 6"	1233	± 7.1	5.42
11' 0"	1264	± 7.4	3.12
23' 6"	1245	± 7.5	1.85

From the tables it can be seen that in all trees there was an increase in fibre length from the base upwards to a certain height, and then a decrease to the top of the tree. As, however, the samples

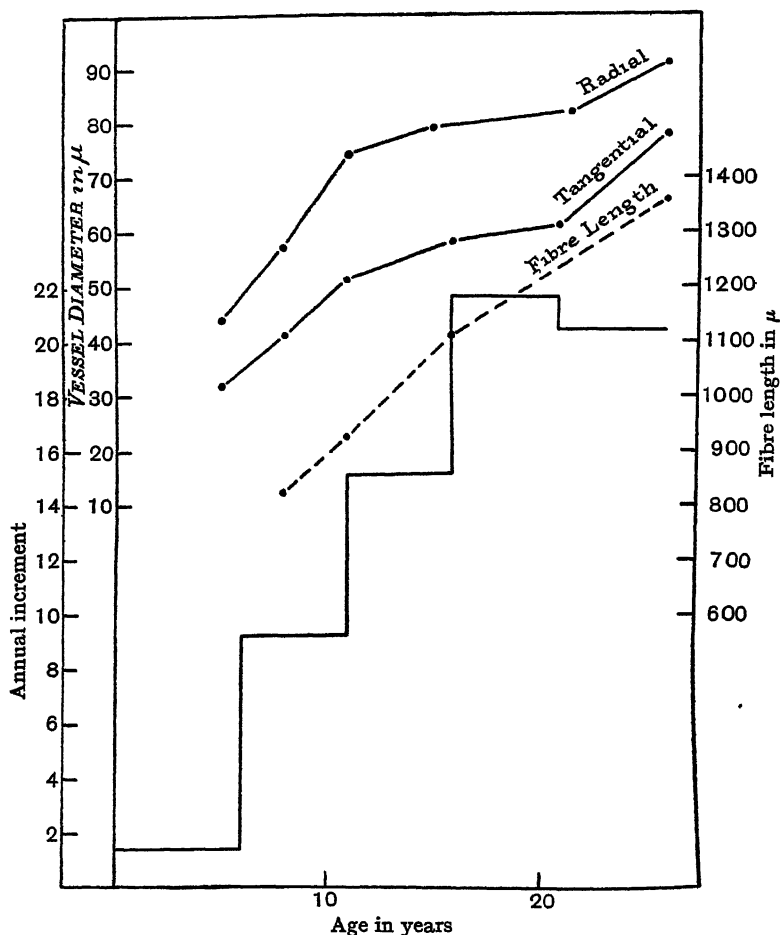


Fig. 5. Birch tree B/I. Fibre length, vessel diameter and periodic mean annual area increment at 4 ft. 3 in. plotted against age.

were taken at intervals of 5 ft. or more it is not possible to state at precisely what height the maximum occurred. The tracheids in the conifers and the fibres in *Quercus rubra* (Eichhorn (7)) show a similar type of variation, but fibre length in the beech (9) and in *Quercus robur* (Hartig (10)) decrease steadily upwards. From a consideration of

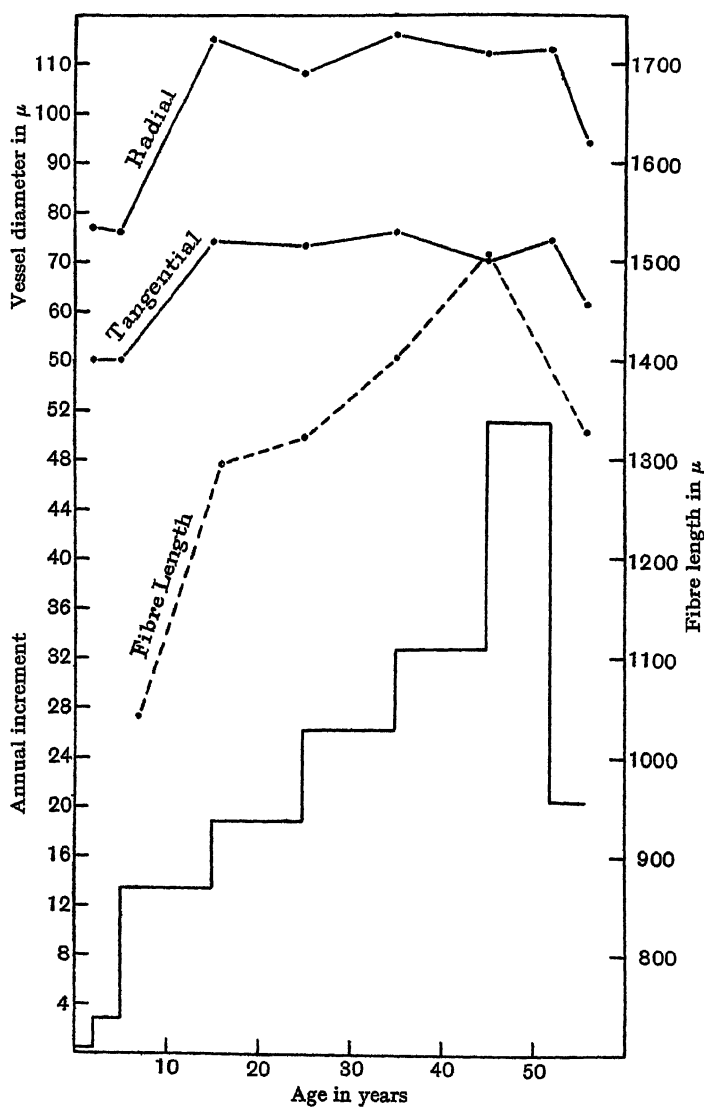


Fig. 6. Birch tree B/IV. Fibre length, vessel diameter and periodic mean annual area increment at the base plotted against age.

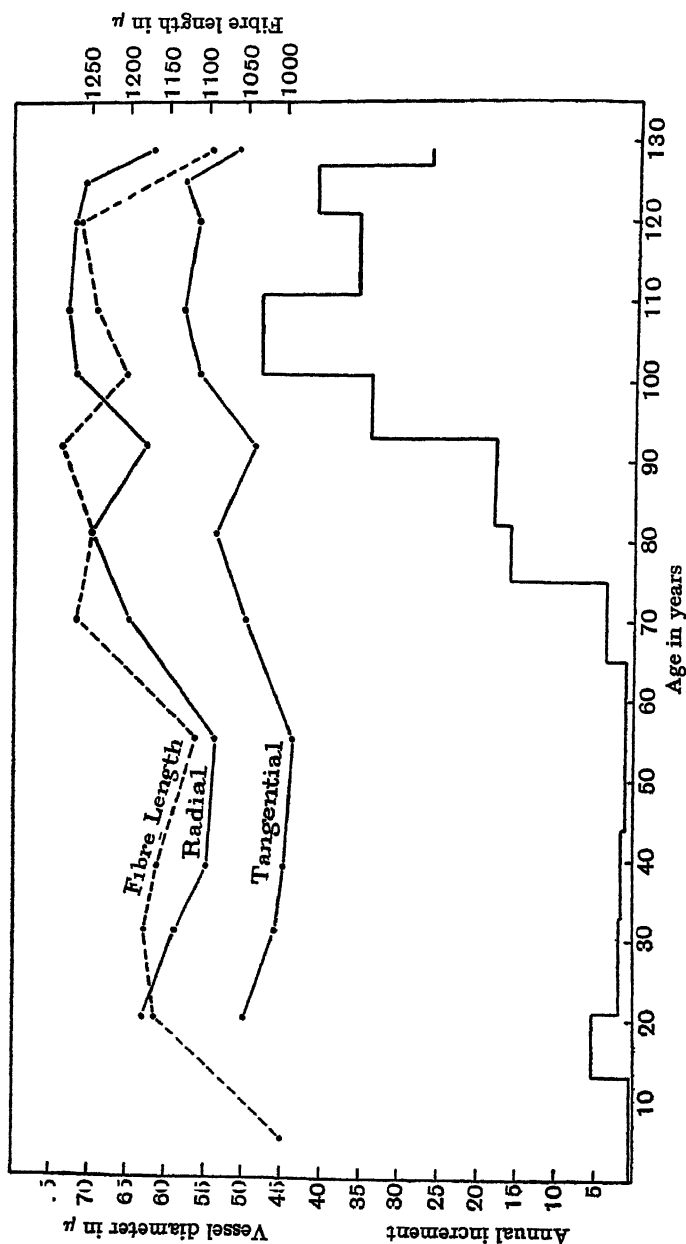


Fig. 7. Beech tree F/II. Fibre length, vessel diameter and periodic mean annual area increment at 6 ft. plotted against age.

Hartig's figures (9, 10), it seems probable that this difference can be ascribed to defects in his methods rather than to inherent differences in the species examined.

It appears, then, that in all trees fibres and tracheids increase in length from the base upwards in the tree and then decrease towards the top. Further Shepard and Bailey (22) and Lee and Smith (15) have shown that the height at which maximum tracheid length

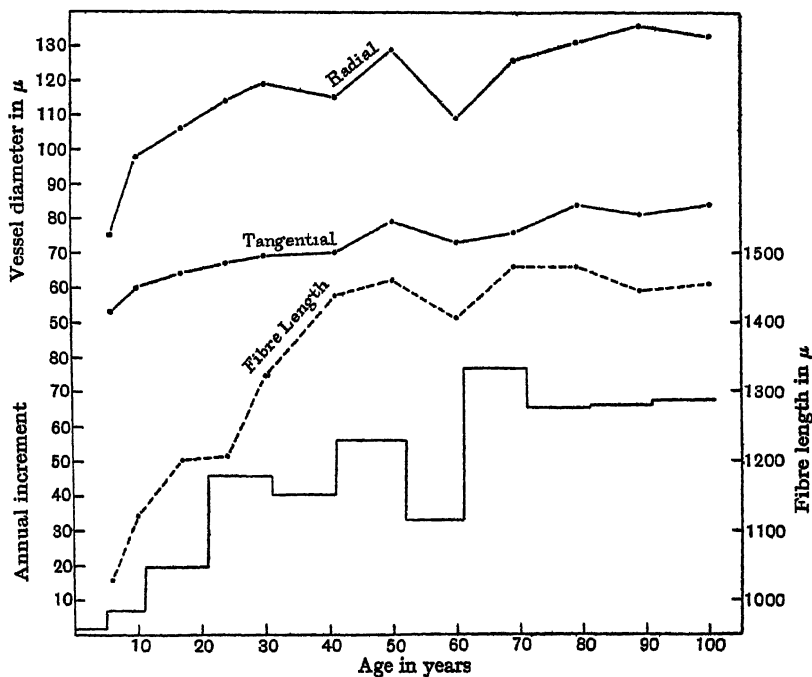


Fig. 8. Poplar tree P/I. Fibre length, vessel diameter and periodic mean annual area increment at the base plotted against age.

occurs in the conifers increases with the distance from the pith, that is to say, as the tree becomes taller. This suggests that the height at which the maximum occurs is related to the total height of the tree, a supposition which would be in accord with Jaccard's ideas on the importance of external factors such as wind in determining the character of the wood in the lower part of the stem. It might then be more illuminating to say that cells decrease in length upwards but that in the lower part of the bole other factors are involved which interfere with this regular behaviour.

(2) *Variation in the proportions of different tissues*

Earlier workers claimed that there was an initial period of rapid increase in cell size corresponding with the youthful stage in the

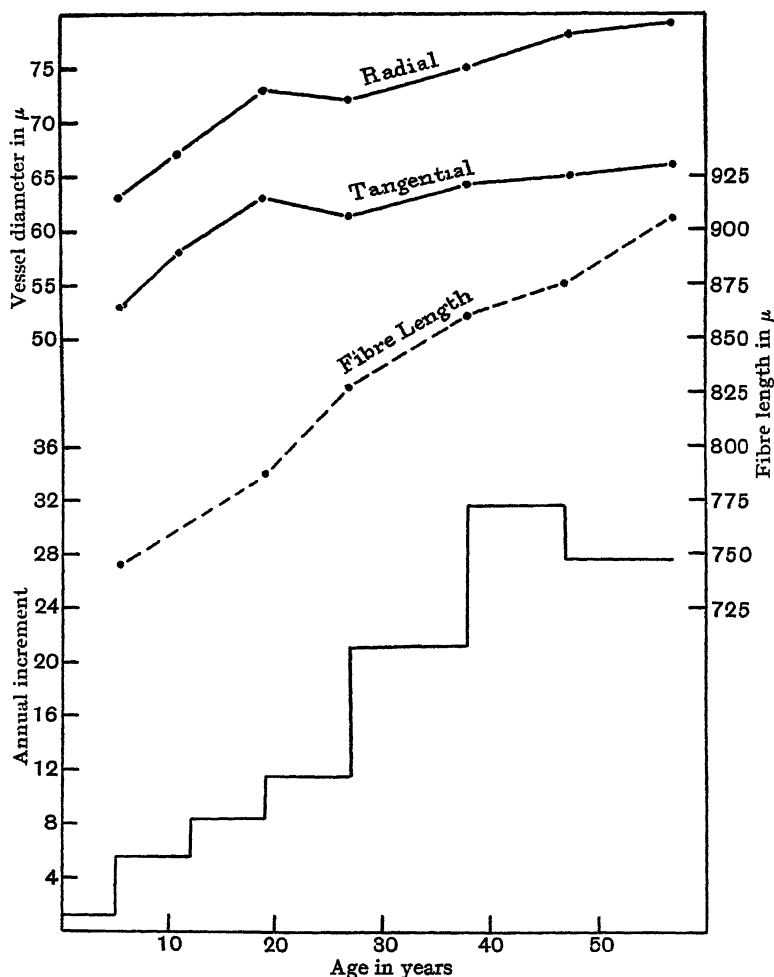


Fig. 9. Sycamore tree S/I. Fibre length, vessel diameter and periodic mean annual area increment at the base plotted against age.

life of the tree and have given figures in support of this idea. In the same way, variations in the proportions of the different tissues have been regarded as dependent on age during this initial period. Hartig(10) observed that the proportion of spring wood vessels, in

the oak, increased outwards each year up to the 40th ring, but from that point the proportion depended on the ratio between the amount

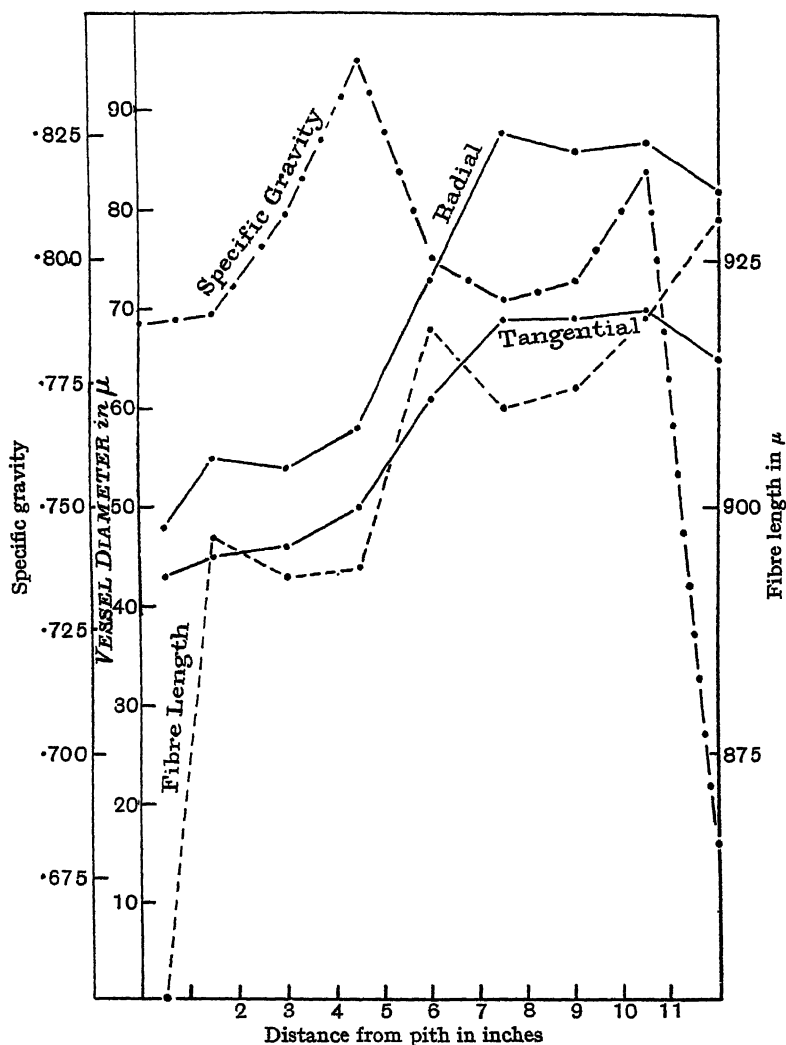


Fig. 10. Rhodesian teak tree T/I. Fibre length and vessel diameter at the base plotted against distance from the pith.

of evaporation and the rate of increment. Conversely, the proportion of fibres was generally greater in youth but was more dependent than the proportion of vessels on the amount of evaporation and the

rate of increment. Hartig suggested further, that in young trees the leaves are more efficient than in older trees and that this leads to an increase in the proportion of mechanical tissue. Clarke's(5) figures for the proportion of spring wood vessels and fibres in elm agree with Hartig's observations as regards the increase in the percentage of spring wood vessels and the decrease outwards in the percentage of fibres. The figures for the percentage of the annual ring occupied by vessels given in Tables XIX and XX show that the changes which occur are by no means as consistent as Hartig's conclusions would lead one to expect.

TABLE XIX

Birch tree B/I. Vessel area as a percentage of the annual ring at different ages

Age in years	1-2	5	10	15	20	25
Vessel area (%)	19.2	14.9	12.2	11.6	11.2	12.6

TABLE XX

Baikiaea tree T/I. Vessel area as a percentage at different distances from the pith

Distance from centre in inches	1½	3	4½	6	7½	9	10½	12
Vessel area (%)	8.5	9.4	12.1	10.7	12.9	13.3	11.9	13.4

The absence in this investigation of any initial period of rapid rise in cell size corresponding to the youthful stage referred to by Hartig made it improbable that any such division could be made when considering changes in the proportions of different tissues. The proportion of the annual ring occupied by vessels at different ages was therefore determined in alder tree A/II (see Table XXI).

TABLE XXI

Alder tree A/II. Vessel area per cent. and number of vessels per sq. mm. at different periods in the life of the tree

Period in years	Vessel area (%)	Vessel number per mm ²			Mean ring width in mm.
		Solitary	Groups	Total	
12-21	13.6	34	18	52	2.30
22-31	16.9	43	17	60	1.65
32-44	20.5	47	22	69	0.70
45-54	12.7	21	11	32	2.15
81-92	20.9	40	18	58	1.50
93-102	21.5	36	14	50	0.97

These figures show, even at an early age, an inverse relation between ring width and the proportion of vessels. The area of the vessels depends both on size and number. Size is approximately proportional to the increment and therefore decreases in a narrow ring, but it will be noticed that the number of vessels per unit area increases very considerably. It appeared that in the diffuse porous woods the total number of vessels in a ring might not be greatly affected in bad years and that consequently the increase in number per unit area (and also in area per cent.) was due to the vessels being more closely packed in a narrow ring. In the elm, Clarke(5) found a high correlation between total spring wood vessel area and ring width, although the proportion of vessels was lower in wide than in narrow rings. In this connection he says "thus it appears that the influence of the 'internal factor' (age) is exerted principally on the dimensions of the elements while the effects of the 'external factors' are seen principally in the varying proportions in which the elements are present." It has been shown that the results of this investigation do not confirm the first part of this suggestion, but, as the figures in Table XXI show, they do agree with the latter part. A more detailed study of the relationship between ring width, the percentage of vessels, and the total number of vessels in the annual ring was undertaken, covering a period of fifteen years from 1911 to 1925 in alder tree A/II. The figures are given in Table XXII below.

TABLE XXII

Alder tree A/II. Variations in ring width, mean vessel diameter, the percentage of vessels, and the total number of vessels in the annual ring for the period 1911-1925

Year	Vessel area (%)	Ring width in mm	Mean vessel diameter in μ		Number per mm. ²	Total number in ring
			Tan- gential	Radial		
1925	25.3	1.08	57	78	67	69,287
1924	20.8	1.28	56	83	47	57,105
1923	24.3	0.78	48	69	65	48,425
1922	17.4	1.41	54	75	43	57,448
1921	34.7	0.40	49	64	100	40,800
1920	24.6	0.96	55	78	55	50,232
1919	22.5	1.69	51	72	58	89,582
1918	21.5	1.66	49	69	60	91,400
1917	22.4	1.33	50	68	58	69,000
1916	24.1	0.86	49	68	64	49,398
1915	18.5	2.00	55	81	43	74,476
1914	23.9	1.02	53	73	62	60,528
1913	21.7	1.46	51	71	60	70,400
1912	15.6	2.92	55	84	32	79,961
1911	23.7	1.31	53	80	55	61,700

The figures for the total number of vessels in the annual ring cannot be regarded as very accurate as small discrepancies in the determination of ring area are multiplied in calculating the total number of vessels in the ring. At the same time they show definitely that although the number increases with increase in ring width the increase is not directly proportional. The increase from the narrowest to the widest ring amounted to some 600 per cent., while the increase in the total number of vessels was only 125 per cent.

Correlation coefficients were worked out between ring width and (a) the percentage area of vessels in the annual ring, and (b) the total vessel area. The percentage area of vessels gave a high negative correlation ($-.823 \pm .056$), while the total vessel area gave a high positive correlation ($+.874 \pm .041$).

The difference in sign between these two correlations may at first sight appear anomalous. It should be remembered, however, that a perfect correlation exists when the percentage variation from the mean of one variable is either directly or inversely proportional to the percentage variation from the mean of the other variable, although the amount of variation in the two variables is not necessarily equal. Thus the difference in sign between the two correlations above is due to the fact that compared with the amount of decrease in the area of any ring, the corresponding decrease in vessel size and total vessel number is comparatively small, so that though the actual area of vessels decreases, its proportion to the area of the ring is increased. The significance of variation in the proportions of different tissues will be considered in the next section of this paper, dealing with variation in specific gravity.

(3) *Variation in specific gravity*

In the ring-porous species Hartig (10, 11), Eichhorn (7), and Clarke (5) state that at a given height in the tree specific gravity is highest at the centre and decreases outwards. In the diffuse porous hardwoods, however, the evidence is conflicting. Stauffer observed that in the birch specific gravity increased steadily from the centre outwards while in the beech Hartig found that it decreased outwards.

An examination of the figures obtained by Hartig (9, 10, 11) and Eichhorn (7) discloses the fact that their data by no means exclusively supported their conclusions, as specific gravity was not always highest at the centre.

A graph (Fig. 11) has been constructed from the data in their tables for a few selected trees, and from the figure it will be seen

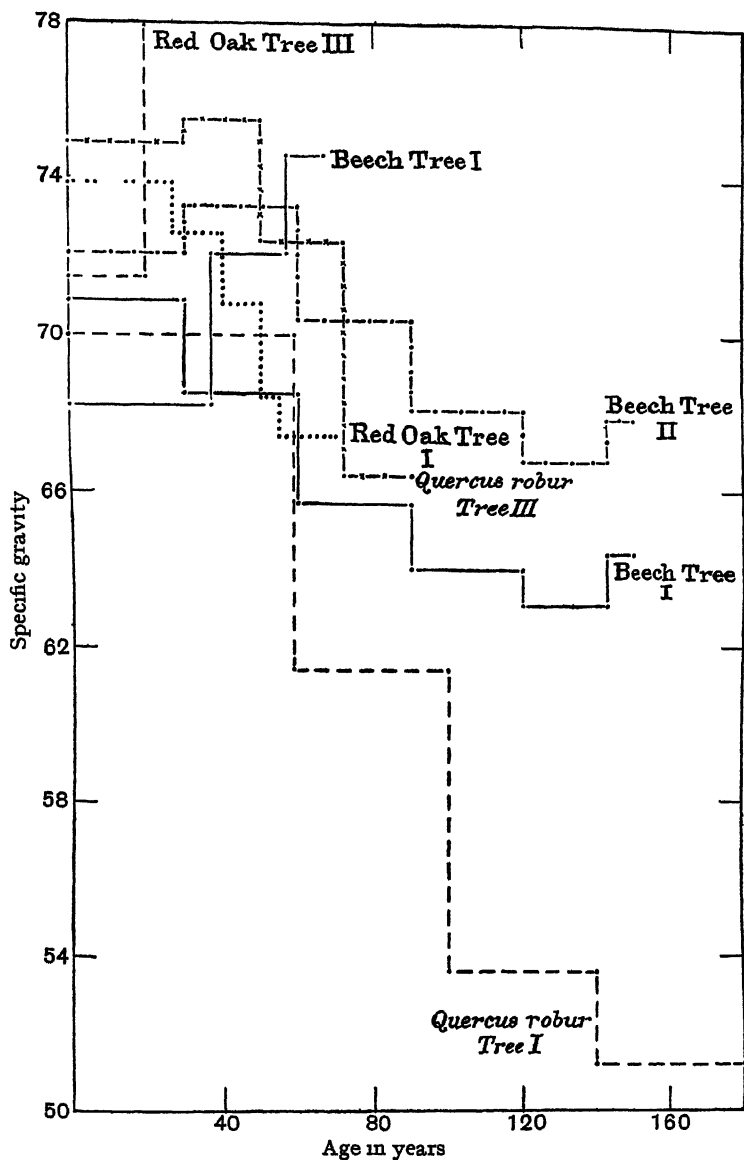


Fig 11 Specific gravity figures, obtained from tables given by Hartig and others, plotted against age. For details see text.

that in some trees specific gravity was low at the centre, then increased for a period, and finally decreased to the outside.

With regard to the variation upwards, Hartig(9, 10, 11, 13) and Eichhorn(7) state that in the same rings specific gravity is highest at the base, decreases upwards in the bole, but increases again in the crown. On the other hand specific gravity has been found to increase upwards in the bole in Canadian poplar (12), in *Eucalyptus saligna* (21), and in some trees of white ash (17).

This investigation has been restricted to diffuse porous woods, so that the most relevant of the above conclusions are Hartig's(9) figures for beech and Stauffer's(23) for birch. The detailed figures for the trees examined in the present investigation are given in Tables XXIII-XXXIII.

It is generally agreed that in the heartwood specific gravity is directly proportional to the amount of wood substance in a given sample. It has been shown that the density of wood substance ($\frac{\text{oven-dry weight}}{\text{saturated volume}}$) is remarkably constant, and this has been determined as being 1.28. The proportion of cell wall to cell lumen varies in different kinds of cells, being highest in the fibres, and lowest in the vessels, hence differences in specific gravity must be primarily determined by anatomical variations affecting the proportion of cell wall to air space. This proportion may be varied by (a) changes in the relative proportions of different tissues, and (b) changes in the relative proportions of wall to lumen in the individual cells. The latter may be affected either by changes in cell size or by changes in the thickness of the walls. Hartig(9) stated that nearly all variations in the weight of wood are associated with the amount of transpiration from the crown, or in other words with the proportion of vessels in the wood. Stauffer(23) on the other hand ascribed increase in specific gravity in the birch to increase in length and wall thickness of the fibres, and Clarke(5) has shown that fibre volume gives the best correlation with specific gravity.

Before considering more fully the effect of different factors on the variation in specific gravity, it may be as well to draw attention to changes in specific gravity observed in this investigation. From Tables XXIII-XXXIII it will be seen that, at a given height, in all trees there was a tendency for specific gravity to be low at the centre, to increase outwards for a period of years and finally to decrease towards the outside. Some exceptions to this were observed, for example, the whole of sycamore tree S/I and the lower discs of

TABLE XXXVI. Alder tree A/IV. Specific gravity and mean ring width at different periods and at different heights in the tree

Base		6' 0"		15' 0"		21' 0"		30' 0"	
1921-1930	.10	.425	.07	.435	.10	.413	.07	.375	.12
1911-1920	.19	.422	.14	.436	.1911-1920	.427	.14	.408	.17
1901-1910	.28	.433	.22	.440	1901-1910	.422	.22	.402	.24
1891-1900	.37	.436	.22	.440	1891-1900	.422	.22	.399	.24
1881-1890	.38	.439	.26	.450	1881-1890	.428	.25	.399	.28
1871-1880	.19	.423	.19	.460	1871-1880	.432	.27	.403	.28
1861-1870	.40	.432	.36	.463	1861-1870	.430	.25	.441	.26
1851-1860	.40	.430	.51	.424	1856-1860	.401	.30	.436	.22
1845-1850	.68	.391	.1848-1850	.50					.386

TABLE XXXVII. Birch tree B/IV. Specific gravity and mean ring width at different periods and at different heights in the tree

Base		5' 0"		11' 6"		17' 0"		25' 0"	
1921-1930	.325	.527	.335	.562	.262	.608	.200	.577	.212
1911-1920	.397	.514	.338	.577	.345	.575	.230	.583	.200
1901-1910	.448	.524	.330	.563	.345	.526	.230	.528	.300
1891-1900	.440	.528	.332	.520	.350	.477	.380	.494	.343
1881-1890	.582	.498	.493	.482	.451	.451	.500	.476	.355
1874-1880	.510	.491	.1875-1880	.540	.468				.461

TABLE XXXVIII. Birch tree B/VI. Specific gravity and mean ring width at different periods and at different heights in the tree

Base		7' 6"		16' 0"		22' 6"		32' 0"	
1926-1930	.460	.560	.270	.590	.260	.525	.260	.580	.310
1921-1925	.370	.559	.280	.605	.230	.563	.360	.551	.559
1916-1920	.300	.576	.260	.591	.250	.532	.270	.520	.270
1911-1915	.370	.540	.400	.553	.290	.594	.450	.491	.552
1906-1910	.440	.526	.498	.498	.520	.483	.533	.470	.320
1901-1905	.390	.496	.1906-1910	.470					.531
1896-1900	.270	.466	1901-1905	.450					

* Sp Gr. = Oven-dry weight
Saturated volume.

TABLE XXIX. *Sycamore tree S/I. Specific gravity and mean ring width at different periods and at different heights in the tree*

Height in tree																	
Base			5' 0"			10' 0"			15' 0"			24' 0"			32' 0"		
Mean		ring width	Mean		ring width	Mean		ring width	Mean		ring width	Mean		ring width	Mean		ring width
Period	in Sp. gr.*		Period	in Sp. gr.*		Period	in Sp. gr.*		Period	in Sp. gr.*		Period	in Sp. gr.*		Period	in Sp. gr.*	
1921-1930	.19	.488	1921-1930	.32	.472	1921-1930	.25	.447	1921-1930	.22	.463	1921-1930	.20	.490	1921-1930	.15	.475
1912-1920	.43	.487	1912-1920	.42	.496	1912-1920	.37	.445	1912-1920	.32	.445	1912-1920	.29	.508	1912-1920	.16	.473
1901-1911	.42	.487	1901-1911	.35	.477	1901-1911	.36	.445	1901-1911	.35	.447	1901-1911	.35	.481	1901-1911	.34	.476
1893-1900	.35	.484	1893-1900	.24	.478	1893-1900	.21	.448	1893-1900	.21	.462	1893-1900	.17	.472	1893-1900	.40	.464
1886-1892	.40	.480	1886-1892	.34	.455	1886-1892	.36	.440	1886-1892	.21	.462	1886-1892	.44	.434	1886-1892		
1881-1885	.51	.478	1878-1885	.31	.466	1882-1885	.22	.442									
1875-1880	.34	.468															

TABLE XXX. *Birch tree B/VII. Specific gravity and mean ring width at different periods and at different heights in the tree*

6' 0"			15' 0"			21' 0"			30' 0"		
Base		Mean ring width	Mean ring width		Mean ring width	Mean ring width		Mean ring width	Mean ring width		Mean ring width
Period	Sp. gr.*	mm.	Period	Sp. gr.*	mm.	Period	Sp. gr.*	mm.	Period	Sp. gr.*	mm.
1921-1930	.244	.498	1921-1930	.207	.520	1921-1930	.170	.554	1921-1930	.180	.530
1911-1920	.197	.528	1911-1920	.197	.533	1911-1920	.207	.525	1911-1920	.240	.517
1901-1910	.355	.525	1901-1910	.285	.504	1901-1910	.253	.511	1901-1910	.300	.525
1891-1900	.246	.556	1891-1900	.263	.505	1891-1900	.263	.487	1891-1900	.198	.488
1881-1890	.280	.556	1881-1890	.242	.502	1881-1890	.200	.470	1881-1890	.242	.471
1871-1880	.275	.485	1871-1880	.308	.447	1871-1880	.367	.443	1871-1880		

TABLE XXXI. *Poplar tree P/III. Specific gravity and mean ring width at different periods and at different heights in the tree*

5' 6"			11' 0"			23' 6"		
Base		Mean ring width	Mean ring width		Mean ring width	Mean ring width		Mean ring width
Period	Sp. gr.*	mm.	Period	Sp. gr.*	mm.	Period	Sp. gr.*	mm.
1921-1930	.0125	.450	1921-1930	.0180	.391	1921-1930	.215	.365
1911-1920	.0445	.453	1911-1920	.0315	.391	1911-1920	.370	.370
1901-1910	.0460	.427	1901-1910	.0405	.399	1901-1910	.425	.432
1891-1900	.0900	.383	1891-1900	.0380	.377	1891-1900	.550	.388
1881-1890	.0990	.366	1881-1890	.0625	.370	1881-1890	.590	.367
1871-1880	.0900	.366	1871-1880	.0730	.368	1871-1880	.590	.361
1861-1870	.1020	.367	1861-1870	.1000	.344	1861-1870		

* Sp. Gr. = Oven-dry weight
Saturated volume.

poplar tree P/II. Variation upwards is much more perplexing and it is difficult to discern any general tendency. For example in the same ring or period of years traced upwards certain well-marked changes in specific gravity can be observed, but these changes are not always parallel in different periods of the same tree. An example of this may be seen in Table XXX, birch tree B/VII, in the 1911-1920 and 1900-1910 periods. In the alders, and two of the birches (IV and V) specific gravity showed two peaks, one a short distance above the base (5 to 7 ft.) and the other just below the crown (20 to 25 ft.). In the sycamore there was a decrease from the base upwards to a height of 10 ft., followed by an increase at 24 ft. and a final decrease at 32 ft. In the poplar specific gravity appeared to behave in a similar manner but no material was available to show whether or not it fell above 23 ft.

TABLE XXXII

Alder tree A/II. Specific gravity and mean ring width at different periods and at different heights in the tree

Period	Height in tree base		Period	Height in tree top	
	Mean ring width in mm.	Sp. gr.*		Mean ring width in mm.	Sp. gr.*
1920-1929	0.97	.372	1920-1929	0.93	.372
1908-1919	1.50	.372	1909-1919	1.32	.372
1891-1907	1.41	.388	1890-1908	1.20	.388
1882-1890	2.17	.402	1880-1889	1.35	.402
1872-1881	2.15	.428	1870-1879	2.60	.428
1859-1871	0.71	.403	1862-1869	2.28	.403
1849-1858	1.65	.415			
1839-1848	2.30	.410			
1831-1838	1.56	.326			

$$* \text{Sp. Gr.} = \frac{\text{Oven-dry weight}}{\text{Saturated volume}}.$$

When the changes in specific gravity were compared with changes in the other features examined, namely, cell size, percentage area of vessels, and annual increment, it was obvious that a perfect relationship with any of these features did not exist. This may be accounted for by the fact that the amount of wood substance in a given sample depends on at least three factors, (a) the proportions of the different tissues, (b) the thickness of cell walls and (c) the size of cells. Since variations in these factors are not necessarily proportional or simultaneous, changes occurring in two of them may be counteracted by the behaviour of the third.

TABLE XXXIII
*Specific gravity and mean ring width at different periods and at
 different heights in the tree*

Period	Poplar tree P/I			Beech tree F/II			Birch tree B/I			Baihiara tree T/I		
	Base			6' 0"			4' 3"			Base		
	Mean ring width in mm.	Sp. gr.*	Period	Mean ring width in mm.	Sp. gr.*	Period	Mean ring width in mm.	Sp. gr.*	Period	Distance from pith	Sp. gr.*	Period
1921-1930	.27	.362	1923-1930	.256	.566	1925-1929	.28	.488	1925-1929	12"	.693	
1911-1920	.31	.376	1913-1922	.270	.570	1913-1922	.58	.492	1920-1924	10½"	.819	
1901-1910	.25	.344	1903-1912	.435	.555	1915-1919	.50	.482	1910-1914	9"	.804	
1891-1900	.35	.403	1895-1902	.400	.572	1910-1914	.70	.477	1904-1909	7½"	.793	
1882-1890	.32	.372	1884-1894	.276	.560	1904-1909	.70	.474		6"	.795	
1878-1881	.68	.358	1877-1883	.321	.564					4½"	.828	
1874-1877	.18	.398	1867-1876	.090	.538					3"	.784	
1871-1873	.53	.385	1846-1866	.026	.546					1½"	.791	
1863-1870	.26	.401	1835-1845	.050	.582					pith	.788	
1861-1862	.55	.375	1823-1834	.062	.573							
1851-1860	.57	.341	1815-1822	.313	.559							
1841-1850	.45	.347	1802-1814	.112	.582							
1835-1840	.38	.333										

* Sp. Gr. = $\frac{\text{Oven-dry weight}}{\text{Saturated volume}}$.

Contrary to Hartig's observations, the percentage of vessels in the annual ring in the first five to ten years was observed to be higher than in the period immediately following, and this fact may account for the low specific gravity at the centre. The subsequent changes would appear to depend on the adjustment of the three variable factors. Towards the outside of the tree, ring width decreases and since it has been shown that there is a negative correlation between ring width and vessel area per cent., the proportion of vessels may be expected to increase outwards. This behaviour agrees with the observed tendency of specific gravity to fall off towards the outside. Although the correlation between the proportion of vessels in the wood and specific gravity is not a high one, yet if there is a marked increase in its porosity the wood must become lighter.

It will be seen from the tables that sudden marked fluctuations in specific gravity generally coincide with marked fluctuations in ring width. Considerable attention in the past has been given to the significance of ring width as a factor affecting specific gravity, and it has been generally stated that broad rings in ring porous hardwoods and narrow rings in softwoods produce wood of the highest specific gravity.

In this connection Cieslar and Janka⁽⁴⁾ investigated a dominant, a co-dominant and a suppressed spruce from two different stands, one rapidly and the other slowly grown. In both stands the dominant trees had wood of lower specific gravity than the co-dominant ones, but in the suppressed trees one was of high specific gravity and the other was of about the same as the dominant of that stand. Hartig⁽¹¹⁾ states however that the width of the annual ring is no criterion of quality. Clarke⁽⁵⁾ has since shown in the elm that the relation between ring width and specific gravity and between the percentage of summer wood and specific gravity was not sufficiently constant to be of value. Paul's⁽¹⁷⁾ conclusions confirm Clarke's results although he pointed out that in the ring porous species a retardation of the rate of growth in diameter brings the rows of large open pores in successive annual rings closer together by reducing the development of that portion of the ring containing thicker-walled summer wood cells. The net result of this is wood with a greater than normal proportion of porous spring wood and a lower specific gravity. In the diffuse porous woods Paul observed that less contrast exists in the portions of the annual ring formed at different periods in the growing season, so that the first gradual retardation of radial growth may not be reflected in the specific gravity of the wood. A continuation

of adverse growth conditions, however, results not only in the formation of narrower rings, but also in the formation of more porous rings, so that the wood becomes correspondingly lighter. In the present investigation instances were found of periods of adverse conditions, as measured by the width of rings, corresponding with reductions in specific gravity.

Several theories have been put forward to account for variation in specific gravity. Hartig(11) and Chancerel(2) maintained that favourable site conditions produce wood of higher specific gravity than less favourable ones; Chancerel also suggested that wood produced in a warmer climate is heavier than that produced upon cooler sites. Cieslar(3) found that spruce grown in the optimum of its natural habitat showed higher lignin content than that grown in locations outside its natural limits of distribution. Paul(17) on the other hand states that in the white ash "the only feature which could be attributed directly to the influence of locality was found in wood from the lower portions of trees cut from the overflow bottom lands along the Mississippi River. This wood was lower in specific gravity than cross sections in the same trees 16 or more feet higher up, in contrast to the wood from all other places where the wood at the base of the tree was heavier than that higher up." Lassila(14), however, in Finland, found a relationship between specific gravity and forest types, which is equivalent to saying that specific gravity varies with variations in the quality of locality. The opinions of Paul and Lassila afford an example of two aspects of this problem; the former has endeavoured to determine the effect of the separate characters of a site and has been unable to show any general correlations between them and specific gravity. Lassila on the other hand regards sites as different only when their productive capacity differs and shows that, regarded in this light, sites do affect the specific gravity of the woods grown on them. This would appear to imply that specific gravity can be controlled by regulating the rate of growth. This is borne out by Paul's(17) observations on the effect of the degree of thinning, which he summarises as follows: "In all the broad-leaved species examined (*Hicoria glabra*, *H. ovata*, *Ulmus racemosa*, *Acer saccharum*, and *Liriodendron tulipifera*) severe crowding in the stands resulted in a decrease in the specific gravity of the wood, while relief from crowding was always accompanied by an increase in specific gravity. In addition, the production of wood of uniformly high specific gravity was concurrent with a well-sustained, usually a fairly rapid, growth rate, so that in future crops

of species, such as ash and hickory, the trees may be brought to merchantable maturity in a comparatively short rotation."

As has been mentioned the figures obtained for specific gravity in the present investigation support these views in so far as periods of marked suppression, as indicated by ring width, coincided with reduced specific gravities. Apart from such periods of marked suppression, ring width is not a useful index of the rate of growth, consequently no attempt has been made to demonstrate any close relationship between ring width and specific gravity. Increment curves (based on one radius only) calculated for certain of the trees examined (Figs. 2-8) show that while both annual increment and specific gravity increase outwards from the centre the increase is not parallel, for specific gravity had reached a maximum, and begun to decrease, before there was any sign of a decrease in the annual increment.

It is felt that more definite conclusions regarding variations in specific gravity cannot be formed without the collection of fuller data. In the first place local fluctuations are accentuated by determining the specific gravity of samples from one radius only. This objection can easily be overcome by taking samples from two or more radii. Secondly annual increment and not ring width should be used to distinguish periods of suppression, and the calculation of increment should be based on more than one radius. Thirdly additional material should be selected to include trees which have suffered marked suppression at different stages in their development.

SUMMARY

1. The relative accuracy of different methods of measuring the saturated volumes for specific gravity determinations were examined. The use of different methods was found not to produce any important differences in the calculated values of specific gravities.

2. The amount of range in cell size in any one sample has been shown to be considerable, and the necessity for basing means on a large number of observations demonstrated. To render differences between the means significant, it was found that the means should be based on not less than 300 measurements.

3. A new method was developed for measuring the proportion of vessels per unit area.

4. Variation in cell dimensions has been studied in two planes, outwards from the pith at a given height in the tree and upwards in the tree in the same annual ring or period of years. A high

positive correlation was found between annual increment and cell size at a given height in the tree; fluctuations in the annual increment as a result of suppression coincided with fluctuations in cell size. Cells were observed to be still increasing in size at 100 years but the material was not sufficiently old to show whether size decreased when the increment ultimately declines.

5. The initial period of rapid rise in cell size lasting for 40 years or more referred to by earlier workers was not observed in the species examined, though a somewhat more rapid rise in cell size in the first 5-10 years did occur, and this period coincided with the period of most rapid increase in annual increment.

6. In the vessels radial diameter as a rule showed a closer correlation with annual increment than tangential diameter. It is suggested therefore that the tangential is better than the radial diameter where cell size is used for purposes of identification.

7. Variation in fibre length at different heights in the tree was found to be in conformity with the variation in tracheid length in the conifers, in that there was an increase in length upwards to a certain height and then a decrease to the top of the tree.

8. Variations in the proportions of vessels in the annual ring were shown to be related to external conditions. A high negative correlation of -0.823 ± 0.056 between the proportion of vessels in the annual ring and ring width was found and a high positive correlation of $+0.874 \pm 0.041$ between total vessel area and ring width.

9. The total number of vessels in the annual ring increased with increase in ring width, but the increase was not directly proportional; the number of vessels per unit area increased very considerably with a decrease in ring width.

10. At a given height in the tree specific gravity tended to be low at the centre and to increase outwards for a period and then to decrease towards the outside. No close relationship was found between specific gravity and cell size, or the proportion of different tissues or annual increment. Although ring width did not show a general relationship to specific gravity, sudden marked fluctuations in ring width generally coincided with fluctuations in specific gravity.

11. Variations at different heights in the tree, in specific gravity in the same annual ring or period of years showed apparent differences between individual trees which could not be reconciled with any general law.

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THE WOOD OF THE STERCULIACEAE

I. SPECIALISATION OF THE VERTICAL WOOD PARENCHYMA WITHIN THE SUB-FAMILY STERCULIEAE

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(With Plates IV and V)

INTRODUCTION

THE Sterculiaceae is a family of tropical and sub-tropical plants, comprising 48 genera and about 600 species, of which many are trees. Systematically it is placed by Engler⁽²⁾ in the Malvales (Dicotyledons, Archichlamydeae), and shows many features in common with the other families of that group. Three genera are economically important—*Theobroma* and *Cola* (fruits) and *Tarrietia* (timber). Many of the arborescent genera give a soft wood which is used locally, and some have a tough bark which is used as fibre.

The arborescent genera are classified as follows by Engler⁽²⁾:

Sub-family 1.	ERIOLAENEAE.	<i>Eriolaena.</i>
„ 2.	FREMONTIEAE.	<i>Cheiranthodendron.</i> <i>Fremontia.</i>
„ 3.	DOMBEYAE.	<i>Melhama.</i> <i>Dombeya.</i>
„ 4.	HERMANNIEAE.	<i>Melochia.</i>
„ 5.	BUETTNERIEAE.	<i>Commersonia</i> <i>Scaphopetalum.</i> <i>Leptonychia.</i> <i>Theobroma.</i> <i>Guazuma.</i>
„ 6.	LASIOPETALEAE.	<i>Thomasia.</i>
„ 7.	HELICTEREAE.	<i>Reevesia.</i> <i>Pterospermum.</i> <i>Kleinovia.</i>
„ 8.	STERCULIEAE.	<i>Sterculia.</i> <i>Brachychiton.</i> <i>Pterygota.</i> <i>Firmiana.</i> <i>Scaphium.</i> <i>Tarrietia.</i> <i>Pterocymbium.</i> <i>Cola.</i> <i>Heritiera.</i>

An investigation of the wood of these genera shows, however, that they do not entirely maintain this relationship when anatomical features are considered instead of floral and morphological ones.

There appear to be two distinct lines of specialisation of storage tissue within the family, one affecting the vertical wood parenchyma, the other involving an elaboration of the tissue of the rays, and culminating in the rays with "tile-shaped" cells (*zeigelstein-formige Zellen* of Moll and Janssonius (6), etc., henceforth abbreviated to tile-cells), which are found only in this and two nearly related families (Tiliaceae and Bombacaceae).

There is, so far as has been ascertained, no wood, either in the Sterculiaceae or in the other two families, in which both types of specialisation occur. There are genera without either specialised rays or broad bands of vertical parenchyma, but—at least in the material available for this investigation—no genera with both these features, which leads to the supposition that they are alternative types of specialisation.

When different woods are examined it is often found that genera which are placed in the same group in a systematic classification are very different anatomically, while, conversely, woods with very similar anatomical features are sometimes placed in quite distinct sub-families.

It seems that some slight rearrangement of the classification of the Sterculiaceae is desirable on anatomical grounds, and the first aim of this investigation has been to establish a satisfactory classification based upon the structure of the wood. In order to do this some decision must be reached as to the relative value of the various features of the wood, and the degree of variability of these features within a single species must be determined.

A study of the differences in the distribution of the tissues within a single genus or group of closely related genera ought to provide sufficient data from which to arrange them in a series, and from this to draw conclusions as to those features which may be considered primitive and advanced.

F. H. Frost (3) has established certain facts with regard to the correlation of length of vessel segments and other features, and has concluded that woods with long segments are less highly specialised than those with short ones. This will provide a means of testing the validity of any series that may be arranged within the Sterculiaceae, for if the variation in segment length shows any agreement with the variation in tissue specialisation it will not only afford a justification

for the series, but will indicate in which direction the series should be read.

The results at present achieved will be reviewed separately under the following headings:

I. Specialisation of the vertical wood parenchyma within the sub-family Sterculieae.

II. Specialisation of the rays. (To follow in a separate paper.)

I. SPECIALISATION OF THE VERTICAL WOOD PARENCHYMA
WITHIN THE SUB-FAMILY STERCULIEAE

Reference has already been made to the two types of specialisation which occur within the Sterculiaceae, and to the fact that the two types are never found in the same wood. Therefore, when the specialisation of the vertical wood parenchyma is under consideration, the genera which have rays with tile-cells can be ignored.

The genera can be arranged, according to their rays, in the following classes:

(a) *With tile-cells:*

Guazuma.	Pterospermum.
Kleinhovia.	Scaphopetalum.
Leptonychia.	

(b) *With sheath-cells* (Hüllzellen of Moll and Janssonius (6), etc.). These are less marked in some genera than in others, but are always present to some extent in some rays:

Brachychiton.	Pterocymbium.
Cheiranthodendron.	Pterygota.
Cola.	Scaphium.
Firmiana.	Sterculia.
Heritiera.	Tarrietia.

(c) *Without sheath-cells, but with heterogeneous rays:*

Commersonia.	Melhania.
Dombeya.	Melochia.
Eriolaena.	Theobroma.
Fremontia.	

The genera without tile-cells can be arranged according to their wood parenchyma as follows:

(aa) *Without broad bands of parenchyma:*

Cheiranthodendron.	Fremontia.
Commersonia.	Heritiera.
Dombeya.	Theobroma.
Eriolaena.	

(bb) *With broad bands of parenchyma; at least in some species:*

Brachychiton.	Pterygota.
Cola.	Sterculia.
Firmiana.	Tarrietia.
Pterocymbium.	

A glance at the classifications on p. 121 will show that the genera included under (b) and (bb) are—except *Cheiranthodendron*—to be found in the sub-family Sterculieae, together with *Heritiera* which has no broad bands of parenchyma in any of the species of which material was available for investigation. This sub-family has been chosen for detailed study because it shows great variation in the distribution of the parenchyma, and it is likely to supply data from which a series of primitive and advanced types can be built up.

But before it is possible to decide upon the relatively primitive or specialised condition of any feature it is necessary to consider the probable origin of such a feature, and also, if possible, to correlate it with some other feature about which more is known.

It is generally believed (Jeffrey(4), Torrey(7)) that the parenchymatous cells of the wood have arisen through the septation of tracheids, and therefore it is reasonable to assume that the more features the cells of the parenchyma strands have in common with tracheids, the more primitive the parenchyma will be; and conversely, that in the more specialised types of parenchyma the cells will differ considerably from tracheids, or may even have lost any features they ever had in common.

The more primitive type of parenchyma might be expected to have rather narrow septate cells, so that the whole strand would show its origin from a single tracheid quite clearly; the extreme ends of the strand should be pointed and the cross-walls straight. More frequent septation would lead to a more advanced condition, and this, together with the resulting shortening of the individual cells of the strand, would tend to diminish the likeness to the original tracheid.

An examination of the woods of the Sterculieae shows that a wide range of parenchyma types can be picked out. A detailed description will first be given of isolated woods, showing examples of the different types of parenchyma strand, and then the remaining species will, if possible, be fitted in to form a series.

Primitive strands are found in *Pterocymbium jaranicum* R. Br., and *Pterocymbium tinctorium* Merril. In these woods the fibres are very thin-walled, and have large lumina, so that it is very difficult, on the cross-section, to distinguish between parenchyma and fibres. *Sterculia villosa* Roxb. (Fig. 1) and *S. crassiramea* Merril are very similar to these, but there is definitely more parenchyma present, and as the fibres have slightly thicker walls it is easier to distinguish the two tissues on the cross-section.

On the tangential section the appearance of the parenchyma is

much the same in all four woods (Figs. 4 and 5). Both fibres and parenchyma are storeyed and the ends are pointed and interlock with one another like a series of gables. The cells of the parenchyma, which are distinguished by their cross-walls, are easy to recognise on the longitudinal sections, but some cells are also present which are wider and thinner walled than the fibres, but are not divided up by cross-walls. These may represent a still more primitive condition, before septation into the component cells of the strand had occurred. The strands are generally formed of only two cells, separated by a thin cross-wall which meets the side walls at right angles without leaving any intercellular spaces (Figs. 4 and 5).

Parenchyma strands which differ markedly from tracheids are found in the species which have broad bands of parenchyma clearly visible to the naked eye on the cross-surface of a wood block. These bands, which are broad enough to include the vessels, are well developed in *Sterculia appendiculata* K. Schum. (Fig. 3) and *S. oblonga* Mast. The fibres and parenchyma are very distinct on the cross-section of these woods, as the fibres are much narrower than the parenchyma cells and have much thicker walls and smaller lumina. On a tangential section the parenchyma strands are seen usually to be formed of four cells, with rounded angles and large intercellular spaces between them. The strands still have marked gable ends which interlock with one another without intercellular spaces (Fig. 6).

There are one or two species of *Sterculia* with broad bands of parenchyma which seem to be different in origin from those mentioned above. *Sterculia pallens* Wall. is an example of this type, in which the bands are less regular and less sharply defined, and appear to have originated as extensions of the paratracheal parenchyma, and not to have any connection with narrow metatracheal lines, which are absent from this wood. *Sterculia pallens* has probably a closer connection with *S. scaphigera* Wall. than with the other members of the genus. The latter has, however, lately been transferred to the genus *Firmiana*, as *F. Wallichii* R. Br. and it is obvious that the position of the species is doubtful. *Sterculia ornata* Wall. is another species which does not appear entirely to fit in the main evolutionary sequence. The wood is very soft and light, and consists almost entirely of parenchyma. The distribution of the fibres and parenchyma can, however, be seen clearly at the limits of the growth rings, and is of the same type as the softer woods such as *S. tragacantha* Lindl. with single lines of parenchyma and fibres alternating. The mass of parenchyma found later in the ring, though apparently normal for the species, probably

represents a side line of development, and has no connection with the well-defined bands found in the more advanced species.

An attempt to arrange the species and genera of the Sterculieae in order, according to their vertical parenchyma, gives the following series:

TYPE A. Very little parenchyma, very slight aggregation into lines one, or occasionally two cells wide; usually two cells per strand; parenchyma very difficult to distinguish from the fibres on the cross-section:

<i>Pterocymbium javanicum</i> R. Br.	<i>Sterculia crassiramea</i> Merril.
<i>P. tinctorium</i> Merril.	<i>S. villosa</i> Roxb.

TYPE B. More parenchyma present round the vessels. Single metatracheal lines quite easy to distinguish from the fibres on the cross-section, and occasionally two or three cells wide in part (Fig. 2). There is a tendency in this type for rows of parenchyma to be localised, so that there are occasional patches of fibres which are almost free from them. Strands of two or four cells:

<i>Brachychiton acerifolius</i> A. Cunn.	<i>Sterculia macrophylla</i> Vent.
<i>Heritiera fomes</i> Syme.	<i>S. ornata</i> Wall.
<i>H. littoralis</i> Dry.	<i>S. rubiginosa</i> Vent.
<i>H. macrophylla</i> Wall.	<i>S. Spangleri</i> R. Br.
<i>H. papilio</i> Bedd.	<i>S. tragacantha</i> Lindl.
<i>Sterculia angustifolia</i> Roxb.	<i>S. urceolata</i> Smith.
<i>S. caribaea</i> R. Br.	<i>Tarrietia cochinchinensis</i> Pierre
<i>S. carthaginensis</i> Cav.	<i>T. javanica</i> Blum.
<i>S. colombiana</i> Sprague.	<i>T. perakensis</i> King.
<i>S. foetida</i> L.	<i>T. simplicifolia</i> Mast.
<i>S. harmanda</i> Pierre.	<i>T. sumatrana</i> Miq.
<i>S. hypochra</i> Pierre.	<i>T. utilis</i> Sprague.
<i>S. javanica</i> R. Br.	

TYPE C. Very few scattered single rows; mainly broad bands of metatracheal parenchyma, and very marked paratracheal patches. The most advanced condition appears to be that in which the bands are broad enough to include the vessels, have almost always four cells per strand, and rounded cells with conspicuous intercellular spaces:

<i>Brachychiton rupestris</i> K. Schum. ¹	<i>Pterygota alata</i> Roxb.
<i>Cola accuminata</i> Schott. and Endl.	<i>P. kamerunensis</i> K. Schum.
<i>C. Buntingii</i> Bak. f.	<i>P. macrocarpa</i> K. Schum.
<i>C. caricifolia</i> K. Schum.	<i>Sterculia appendiculata</i> K. Schum.
<i>C. cordifolia</i> R. Br.	<i>S. Blumei</i> G. Don.
<i>C. heterophylla</i> Schott. and Endl.	<i>S. coccinea</i> Roxb.
<i>C. laterita</i> K. Schum.	<i>S. elegantiflora</i> Hutch. and Dalz.
<i>C. laurifolia</i> Mast.	<i>S. oblonga</i> Mast.
<i>C. mirabilis</i> A. Chev.	<i>S. quinqueloba</i> K. Schum.
<i>C. nitida</i> A. Chev.	<i>S. rhinopetala</i> K. Schum.
<i>C. verticillata</i> Stapf.	<i>S. urens</i> Roxb.
<i>Firmiana fulgens</i> K. Schum.	<i>Tarrietia argyrodendron</i> Benth.
<i>F. populifolia</i> Terrac.	

¹ The Australian "bottle tree," a curious member of the family with a much swollen stem with hollow chambers which contain a watery fluid.

It has already been suggested that the degree of subdivision of the strands, and the presence or absence of intercellular spaces may be related in some way to the distribution of the parenchyma. In Diagram 1 the various features of the wood are compared with one another in order to test this hypothesis. The species are arranged according to the type of parenchyma that they show, and according to its distribution, as seen on the cross-section (column 1), and the other details for comparison are to be found in columns 2, 3, and 4.

Column 2 shows the presence or absence of intercellular spaces, and in column 3 the usual number of cells per strand is given, while the less common number is put in brackets. It will be seen that intercellular spaces are entirely absent from types A and B, while they are almost universally present in type C. The two-celled strands which occur in this type are only on the edges of the broad bands; the more centrally placed strands have always four cells.

In his paper on the origin of the vessel in dicotyledons, F. H. Frost (3) suggests that in the more primitive woods the vessel segments are long, the end walls oblique and the perforations often scalariform. As a family, the Sterculiaceae is moderately highly specialised, and it is not surprising to find that the end walls of the segments are nearly all horizontal, the perforations all simple and the segments all relatively short. The segments of the vessels of the various woods were measured in the following way: A Leitz 2 in. objective and a No. 4 eyepiece were used. The end of a vessel segment, on a longitudinal section (radial or tangential), was moved to a definite point on the microscope field and a reading taken on the scale of the mechanical stage. The slide was then moved by the mechanical stage until the top of the segment at the other end of the same vessel reached the fixed point in the field. A second reading of the scale gave the total length of vessel under consideration, and by counting the number of segments the average segment length for that vessel was obtained. The figures must not be taken as representing absolute values, as they are not all from mature material, but they give a clear indication of the approximate length of the segments. If long pieces of vessel are available this method is very accurate. If 25 segments are measured the extreme variation between the means of different groups of 25 for the same wood sample is 1 per cent., on 50 segments it is 0.5 per cent. The number of segments which were measured is given in column 4 (b), and it will be seen that in only 10 cases was it less than 50, and that usually it was more than 100, often many more. Where more than one wood specimen of any species was available slides were cut from each specimen, so that as wide a range of structure as possible might be

included. The agreement between the measurements from such different slides is very marked especially when the inevitable differences between different trees, is considered. Column 4 gives the segment length and column 4 (b) the number of segments on which it is based.

These results are shown again in Diagram 2, from which the general conclusions can more easily be drawn. Types A, B and C refer to the parenchyma classes mentioned above, the numbers represent the segment lengths in μ and the genera are placed as nearly as possible in their right places according to the length of the vessel

DIAGRAM 1

1 Parenchyma types	2 Inter-cellular spaces	3 Cells per strand	4	
			(a) Segment length	(b) No. measured
TYPE A				
<i>Pterocymbium tinctorium</i>	—	2 (-4)	560	12
<i>Sterculia crassiramea</i>	—	2 (-4)	523	208
<i>Pterocymbium javanicum</i>	—	2 (-4)	479	129
<i>Sterculia</i> sp. 5523*	—	2 (-4)	450	58
<i>S. villosa</i>	—	2 (-4)	425	391
<i>S. sp.</i> 1905*	—	2 (-4)	405	121
<i>S. sp.</i> 5517*	—	2 (-4)	404	52
TYPE B				
<i>Sterculia</i> sp. 5531*	—	2 (-4)	423	64
<i>S. caribaea</i>	—	2 (-4)	415	953
<i>S. sp.</i> 5716*	—	2-4	413	37
<i>Tarrietia javanica</i>	—	2 (-4)	427	258
<i>T. sp.</i> 5651*	—	2-4	421	33
<i>T. simplicifolia</i>	—	2 (-4)	400	155
<i>T. perakensis</i>	—	4	393	127
<i>Heritiera papilio</i>	—	4	387	95
<i>Tarrietia sumatrana</i>	—	4	379	100
<i>T. cochinchinensis</i>	—	2 (-4)	364	117
<i>Sterculia harmanda</i>	—	2-4	410	259
<i>S. foetida</i>	—	4	380	259
<i>S. angustifolia</i>	—	2 (-4)	386	217
<i>Brachychiton acerifolius</i>	—	2-4	399	152
<i>Sterculia javanica</i>	—	2-4	395	139
<i>S. ornata</i>	—	2-4	379	217
<i>S. tragacantha</i>	—	(2-) 4	373	345
<i>S. colombiana</i>	—	4	350	89
<i>S. sp.</i> 5518*	—	2 (-4)	377	39
<i>S. macrophylla</i>	—	4	349	103
<i>S. carthaginensis</i>	—	2-4	345	633
<i>S. urceolata</i>	—	(2-) 4	386	39
<i>S. rubiginosa</i>	—	2 (-4)	370	489
<i>Tarrietia utilis</i>	—	2 (-4)	311	1423
<i>T. sylvatica</i>	—	(2-) 4	329	224
<i>Heritiera littoralis</i>	—	2 (-4)	318	273
<i>H. fomes</i>	—	2 (-4)	283	273
<i>H. macrophylla</i>	—	(2-) 4	351	39
<i>Sterculia Spangleri</i>	(+)	4	380	56
<i>S. hypochra</i>	—	2 (-4)	329	85

DIAGRAM I (continued)

I Parenchyma types	2 Inter-cellular spaces	3 Cells per strand	4	
			(a) Segment length	(b) No. measured

TYPE C				
Brachychiton rupestris	—	2 (-4)	256	62
Sterculia quinqueloba	(+)	4	377	299
S. urens	(+)	4	299	350
S. sp. 4441*	—	4	340	55
[Tarrietia argyrodendron	(+)	(2-) 4	377	271]
Pterygota alata	(+)	(2-) 4	324	274
P. macrocarpa	+	4	343	64
Firmiana populifolia	—	4	353	43
Cola sp. 5708*	(+)	4	347	61
Sterculia Blumei	(+)	4	342	21
S. coccinea	—	4	287	124
Cola sp. 5709*	+	4	364	93
Firmiana fulgens	+	(2-) 4	344	298
Cola lateritia	+	4	358	70
C. acuminate	+	4	300	441
C. verticillata	+	4	265	43
C. nitida	+	4	307	128
C. laurifolia	+	4	303	60
Sterculia rhinopetala	—	(2-) 4	325	232
Pterygota kamerunensis	+	4	323	22
Cola Buntingii	+	4	357	125
C. cordifolia	+	4	331	228
C. mirabilis	+	4	324	54
C. heterophylla	+	4	326	80
C. caricifolia	+	4	299	82
Sterculia appendiculata	+	4	346	106
S. elegantifolia	+	4	282	45
S. oblonga	+	4	272	354

The less usual number of cells per parenchyma strand is surrounded by brackets, which are also used to indicate that intercellular spaces are occasionally, but not universally present. An asterisk * indicates that the specific name is unknown, and the material is given the number of the Imperial Forestry Institute collection.

segments. There is inevitably a considerable amount of overlapping between the segment lengths of the classes, especially between types B and C. If *Tarrietia* and *Heritiera*, however, are temporarily excluded, and justification for such a proceeding will be given later, the overlapping is considerably reduced, and it is clearly seen that the remaining five genera form a series ranging from primitive to advanced parenchyma types.

Frost's conclusions concerning vessel segments afford the necessary standards by which this series may be judged. He has proved that the longer segments are found in the more primitive woods. Among the Sterculiaceae the woods with the longest vessel segments are those which also have fine lines of metatracheal parenchyma, predominantly two cells per strand and no intercellular spaces; those

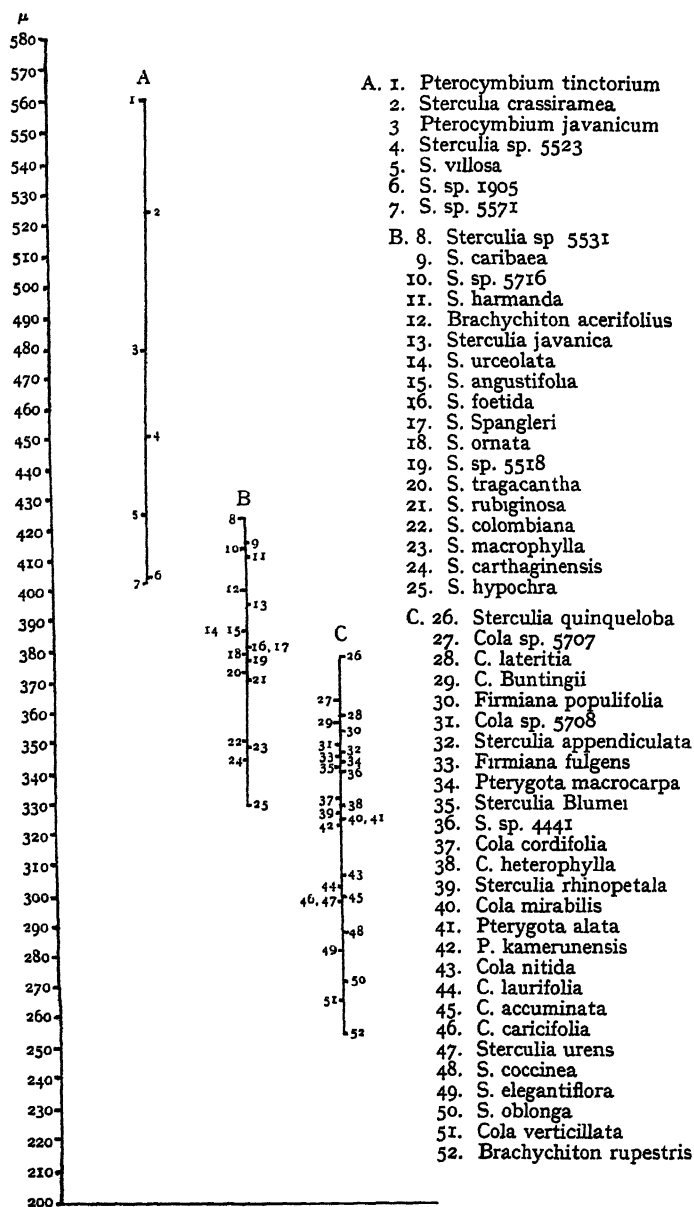


DIAGRAM 2

with the shortest vessel segments have broad bands of metatracheal parenchyma, predominantly four cells per strand, and these cells are often rounded in shape, with intercellular spaces between them.

Tarrietia and *Heritiera* differ from the rest of the Sterculieae in having vessel segments which are unexpectedly short for the type of parenchyma they show. That is to say, if the parenchyma type alone is considered, the wood appears rather primitive; if the segment length alone is considered they appear rather advanced. The parenchyma is arranged in narrow metatracheal lines, usually one, occasionally two cells wide, and each strand often consists of two cells, without intercellular spaces. More specialised strands are, however, occasionally present among these relatively primitive ones. These are much subdivided by cross-walls into almost isodiametric chambers, and within each chamber is a large solitary crystal. This is undoubtedly a more specialised type of parenchyma and is further removed from the ancestral tracheid than the two-celled strand, so that it is not surprising to find that the woods containing it show rather shorter vessel segments than others of their class. These chambered, crystal-bearing parenchyma cells occur only in a few of the most advanced of the other Sterculieae, i.e. in *Sterculia rhinopetala*, *S. appendiculata*, *S. elegantifolia* and *S. oblonga*. In these woods they are rather common and occur usually in the strands at the extreme edges of the metatracheal bands.

It is clear therefore that there is some justification for the separation of *Tarrietia* and *Heritiera* from the rest of the Sterculieae. Possibly they should be excluded altogether from this sub-family on account of these differences in the wood parenchyma. Such a change is also desirable upon morphological grounds, and will be discussed at a later date when the classification of the whole family is reconsidered.

The conclusions to be drawn from this study are that within the sub-family Sterculieae broad bands of metatracheal parenchyma have gradually been developed from narrow lines one or two cells wide. These bands are usually formed of four-celled strands. The upper and lower cells of the strand have pointed ends which interlock and form the zigzag lines usually associated with storeyed parenchyma (Beijer(1), Janssonius(5)) while the intervening cells have a somewhat rounded outline, leaving marked intercellular spaces at the corners. The advanced nature of this type of parenchyma is borne out by the length of the vessel segments, since all the woods of this type have shorter segments than those with narrow lines of two-celled strands.

Heritiera and *Tarrietia* are exceptional genera, for they have, ex-

cept in the case of *T. argyrodendron*, narrow lines of parenchyma, often in two-celled strands and rather shorter vessel segments than would be expected. In these two genera, however, another type of specialisation is to be found, namely the formation of chambered,

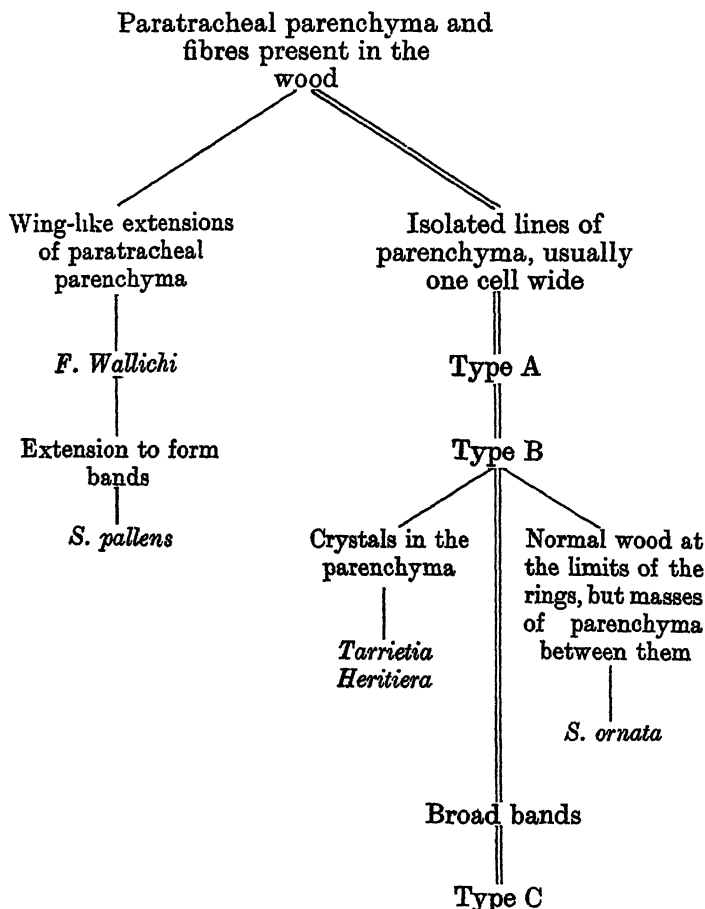


DIAGRAM 3. Suggested lines of evolution of the different parenchyma types within the Sterculiaceae

crystal-bearing parenchyma, which may be considered a secondary specialisation, alternative or supplementary to the main type. It is possible that these two genera should not be placed in the same sub-family as the rest of the Sterculiaceae.

A schematic diagram of the possible relationships within the sub-family is given in Diagram 3.

In conclusion the author wishes to express her thanks to Dr L. Chalk, at whose suggestion this investigation was undertaken, and under whose guidance it has been carried out; to Dr J. Burt Davy, who kindly checked the botanical names of the specimens; and to the following, from whom material has been received: Prof. G. Bredeman, Institut für angewandte Botanik, Hamburg; Prof. A. Chevalier, Musée d'Histoire Naturelle, Paris; J. Collardet, Institut du Comité Nationale des bois coloniaux, Paris; Dr F. W. Foxworthy, Forest Research Institute, Kepong; Prof. H. H. Janssonius, Kolonial Institute, Amsterdam; Prof. S. J. Record, Yale School of Forestry; the Director, Forest Products Research Laboratory, Princes Risborough; the President, Forest Research Institute, Dehra Dun; the Director, Royal Botanic Gardens, Kew.

SUMMARY

1. An investigation of the arborescent genera of the Sterculiaceae shows that the existing classification of the family needs revision.

2. There are two distinct lines of specialisation within the family, the one affecting the vertical wood parenchyma, and the other involving an elaboration of the tissues of the rays.

3. The distribution of the wood parenchyma in the Sterculieae shows great variation, and this sub-family has therefore been chosen for special study.

4. There is a range from species with little parenchyma in short tangential lines, one or two cells wide, which are difficult to distinguish under a microscope, to broad concentric bands which include the vessels and are visible to the naked eye.

5. The strands comprising the former are typically long and two-celled, without intercellular spaces between the cells. Those of the latter are typically four-celled, shorter strands, the individual cells often rounded at the corners, leaving intercellular spaces.

6. The woods with narrow lines of parenchyma have usually longer vessel segments than those with broad bands.

7. *Heritiera* and *Tarrietia* are different in many respects from the other genera of the Sterculieae, though they agree with one another, and they should not be placed in this sub-family.

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EXPLANATION OF PLATES

PLATE IV

- Fig. 1. Transverse section of *Sterculia villosa*. Showing narrow lines of parenchyma. Type A $\times 100$.
 Fig. 2. Transverse section of *S. foetida*. Type B $\times 100$.
 Fig. 3. Transverse section of *S. appendiculata*. Showing broad bands of parenchyma. Type C $\times 50$.

PLATE V

- Fig. 4. Longitudinal tangential section of *Pterocymbium javanicum*. Showing long two-celled strands of parenchyma. Type A $\times 155$.
 Fig. 5. Longitudinal tangential section of *S. villosa*. Type A $\times 155$.
 Fig. 6. Longitudinal tangential section of *S. appendiculata*. Showing short four-celled strands and rounded cells. Type C $\times 155$.

Since going to press material of the following species of *Sterculia* has been received from the Director, Bureau of Forestry, Manila:

S. oblongata R.Br. without intercellular spaces, 2-4 cells per parenchyma strand, segment length 392μ ; type B.

S. philippinensis Merr. without intercellular spaces, 2-4 cells per parenchyma strand, segment length 378μ ; type B.

S. blancoi Rolfe with intercellular spaces, 4 cells per parenchyma strand, segment length 365μ ; type C.

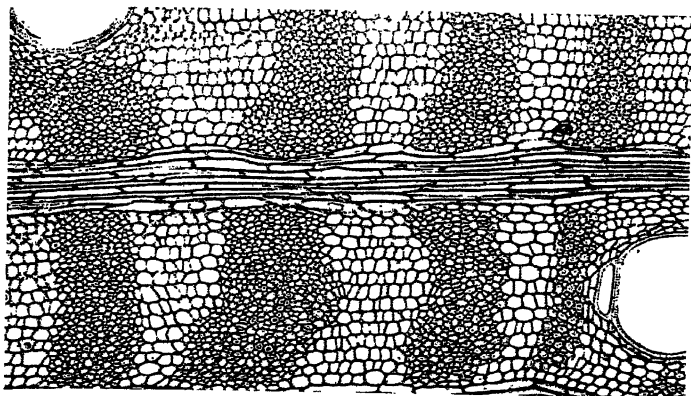


Fig. 3

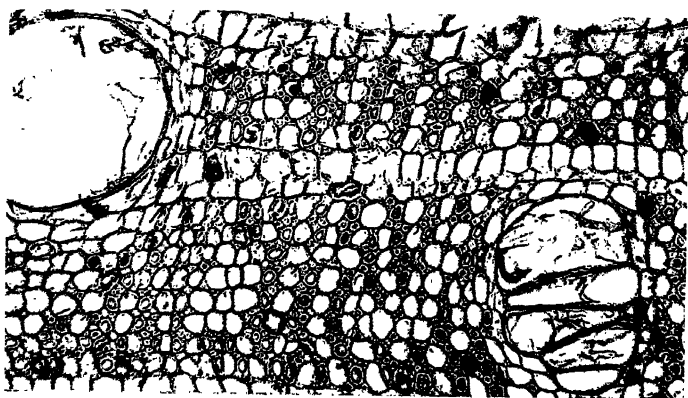


Fig. 2

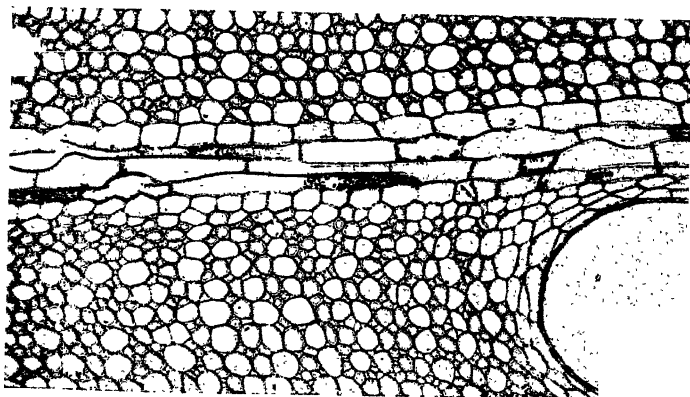


Fig.

CHATTAWAY—THE WOOD OF THE STERCULIACEAE

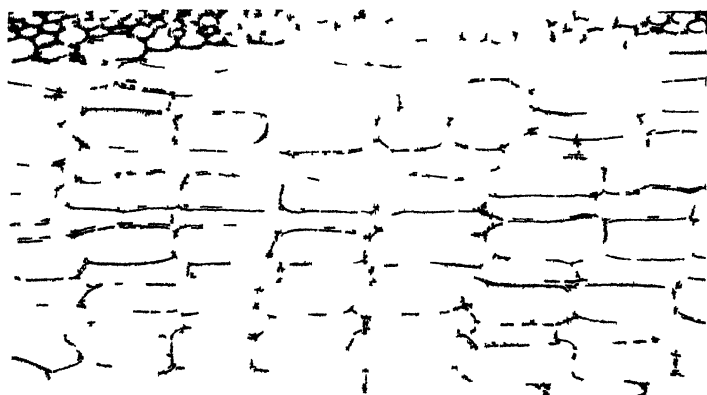


Fig 6

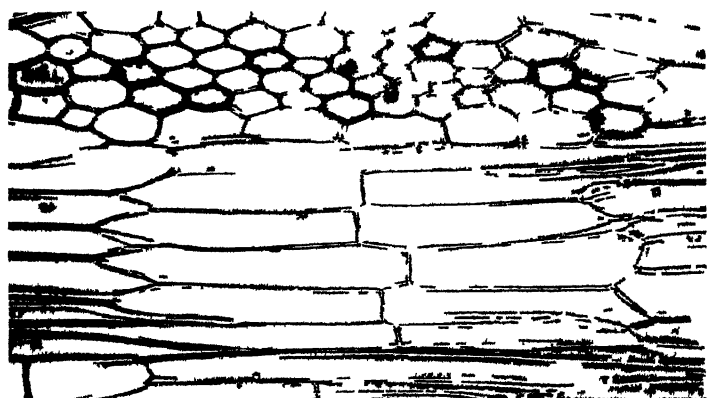


Fig 5

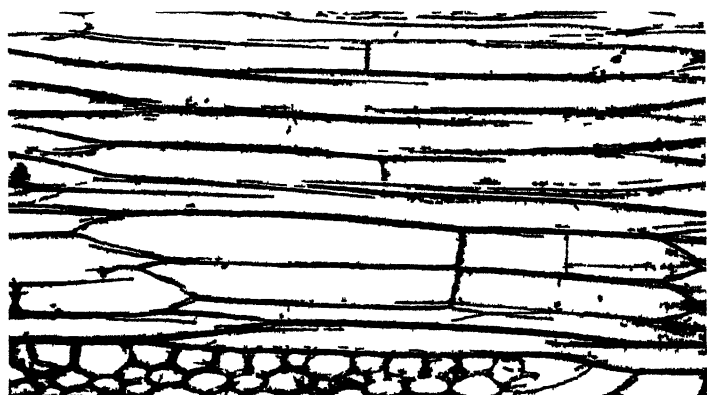


Fig 4

CHATTAWAY—THE WOOD OF THE STERCULIACEAE

AN ACCOUNT OF SOME UNCOMMON BRITISH SPECIES OF THE CHYTRIDIALES FOUND IN ALGAE

BY W. R. IVIMEY COOK, B.Sc., PH.D., F.L.S.

(With 45 figures in the text)

ALTHOUGH a very large number of species belonging to the Chytridiales have been described, many have only been recorded from a single foreign locality. Our knowledge of the group is due very largely to the work of older authorities, and in particular to Zopf (16) in Germany and Atkinson (2) in the United States. In this country published records show that only a relatively small number of those which occur in the algae have been found. From time to time the author has come across various species while examining algae in the course of class work, and it seemed to him desirable that some brief record of these species should be published.

The following species will be considered in this paper: *Woronina polycystis* Cornu, *Rhizophyidium ampullaceum* (A. Br.) Schröter, *Rhizophyidium globosum* (A. Br.) Schröter, *Rhizophyidium transversum* (A. Br.) Fischer, *Rhizidium appendiculatum* Zopf, *Lagenidium gracile* Zopf and *Lagenidium Rabenhorstii* Zopf.

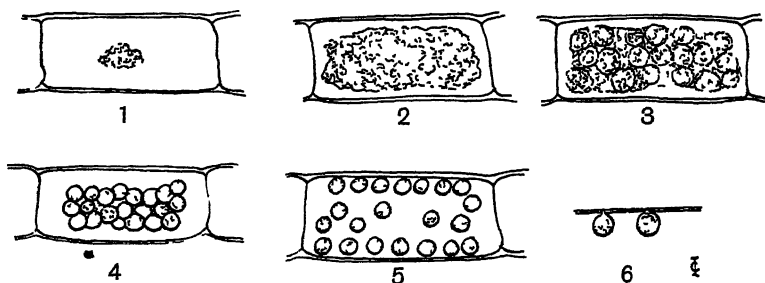
WORONINA POLYCYSTIS Cornu

The organism was found in the cells of *Oedogonium crassusculum* var. *idiosporium* Norst et Wittr.¹ The material was collected in the neighbourhood of Rhythfyn, near Aberystwyth in July, 1926, and was supplied in a fixed condition by the Botanical Supply Station. Unfortunately it was not possible to obtain any fresh material and therefore a study of the organism in a living condition could not be made.

The first stage observed was the appearance in the host of a mass of protoplasm (Fig. 2), resembling the myxamoeba of the Plasmodiophorales. The size of these plasmodia varied, but as a general rule they filled the cell. They were scattered all through the filaments, though

¹ I am indebted to Prof. F. E. Fritsch for identifying the species of *Oedogonium*.

where they occurred in the oogonia the fungus was never in such an advanced condition as in the vegetative cells. This plasmodium was composed of a homogeneous mass of cytoplasm containing a large number of nuclei. The nature of the fixative, however, did not allow of any cytological study being made. When the plasmodium is fully grown the protoplasm becomes segmented into a number of uninucleated parts (Fig. 3); and around each a spore is formed (Fig. 4). As these spores mature they were found to become orientated around the periphery of the cell of the host (Fig. 5). Finally from each spore a zoospore emerged. There was no evidence that more than a single zoospore was liberated from each spore. In some examples it was possible to see that a beak was produced enabling the zoospore more easily to escape through the wall of the host cell (Fig. 6). Owing to



Figs 1-6. *Woronina polycystis*.

1. Young plasmodium 2. Mature plasmodium. 3. Fragmentation of plasmodium into spores. 4. Spores 5. Migration of spores to periphery of cell. 6. Papillae developed on mature spores for the emission of zoospores.

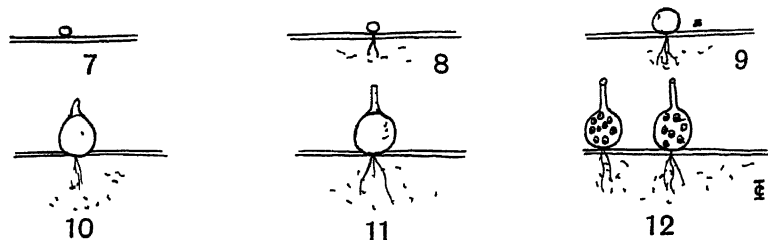
the nature of the material no further study of the zoospores could be made, but in a few cells very small amoeboid masses were found (Fig. 1), and it is concluded that these represent an early stage in the development of the fungus after the zoospore has again penetrated the host. No evidence of fusion of the zoospores was obtained.

Woronina polycystis was described by Cornu (5) in 1872, who found it in the cells of *Saprolegnia spiralis* and in certain species of *Achlya*. In the same paper he gives an account of *Chytridium glomeratum* which differed from *W. polycystis* in having spiny spores. Later Fischer (8) transferred this organism to the genus *Woronina* under the name *W. glomeratum*. This latter species occurred in the cells of species of *Vaucheria*. A third species *W. elegans* was described by Perroncito (11) in 1888, and differed chiefly in having a Rotifer for a host. In 1889 Dangeard (6) made a further study of the genus in the

course of his work on the lower fungi. Of these three species *W. polycystis* is the most common, and also the most completely described. It differs, however, from the material I have examined, in the nature of the host plant, which is normally one of the Saprolegniaceae, whereas I have found it in a species of *Oedogonium*. An additional type of reproduction by cystosori, which originate by the aggregation of resting spores, has also been described. No such structures occurred in my material, but as this is apparently an over-wintering organ this was not surprising since the material I had was collected in mid-summer. The chief difference therefore is the host, and I feel there is insufficient evidence to regard this as a new species on those grounds, especially as the size of the spores is approximately the same as that already given¹. It is more probable that this is a record of an additional host in which *W. polycystis* can occur.

RHIZOPHYDIUM AMPULLACEUM (A. Br.) Schröter

This organism was found on the same material of *Oedogonium crassusculum* var. *idiosporium* Norst et Wittr. as the last species. It lives almost exclusively as an ectoparasite and only the smallest mycelial threads penetrate into the host cell. It was possible, how-



Figs. 7-12. *Rhizophydium ampullaceum*.

- 7 Infection of host. 8. Growth of fungus and development of absorption hyphae. 9 Nearly mature zoosporangium. 10. Zoosporangium with beak developing. 11. Zoosporangium with mature beak. 12. Zoosporangium with zoospores and beak perforated at the top.

ever, to make out these hyphae and to see that it was by their aid that food was obtained from the host cells. The earliest stage found consisted of a very small spherical mass of protoplasm which settled down upon the wall of the host cell (Fig. 7) and sent out a tiny hypha which entered the host (Fig. 8). This hypha at a later stage was seen

¹ Mr. A. W. Bartlett considers that the difference in host plant is sufficient grounds for separating the organism as a new species.

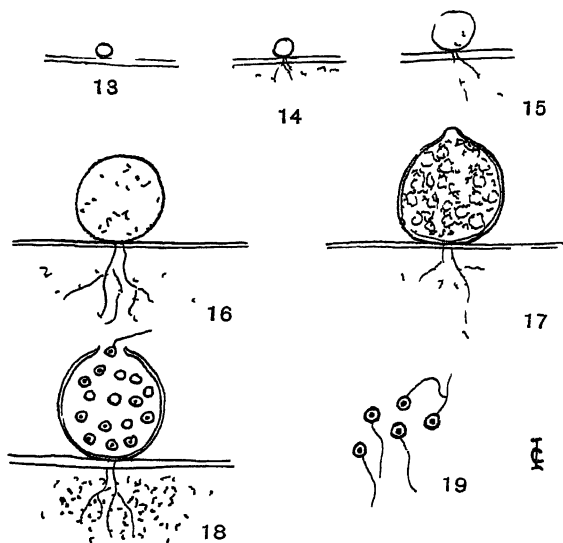
to become branched and to ramify in the cytoplasm of the host cell. As the organism grows the exterior part of the organism becomes enlarged and a wall is formed which stains up clearly (Fig. 9). This swells up and it can be seen that the protoplasm within is becoming more granular. The mature sporangium formed in this way measured 6μ in diameter. Later a swelling appears at the apical end which forms a short straight tube (Figs. 10, 11) and finally the contents divide up into a number of zoospores (Fig. 12). I was unable to observe any flagellum upon these structures, since none of them was in a living condition.

Rhizophydium ampullaceum was first described by A. Braun (3) in 1855 under the name *Chytridium ampullaceum*, and was later transferred to the genus *Rhizophydium* by Schroter (13). Although several other species have been studied in detail, particularly by Atkinson (2), Martin (9), Coker (4) and Melhus (10) in America, no recent account of this species has been published. Atkinson recorded its occurrence in Freeville, New York, but did not give an account of the organism. The organism has been found in various species of *Oedogonium* and also in *Mougeotia*.

RHIZOPHYDIUM GLOBOSUM (A. Br.) Schröter

This species was found in material of *Ulothrix* which was sent to me from The University, Sheffield. It was collected in Graves Park, Sheffield in January, 1931. It is by no means uncommon in algal material and several accounts of it have been published. The species resembles the last in the general development, the zoospore settling down upon the host filament (Fig. 13) and then sending out fine hyphae which enter the host cell (Fig. 14) and obtain food from it. The zoospore itself enlarges and becomes swollen out into a zoosporangium (Figs. 15, 16), which in the specimens I examined measured up to 50μ in diameter. When mature the tip of the sporangium becomes only very slightly beaked (Fig. 17) and the zoospores escape through the aperture (Fig. 18). These zoospores (Fig. 19) were easily seen passing out from the zoosporangium and each zoospore measured $2-3\mu$ in diameter. They were spherical and possessed a single flagellum. Since I was able to examine fresh material it was possible to see the rapid escape of these swarm spores, the whole mass being emitted from the zoosporangium within quite a short time. This process was described in detail by Atkinson (1) in 1894. After a period of activity the zoospores settle down on the surface of the host filament and presumably start to re-infect the host. Although I could not find any

definite evidence in the material I examined I was told that it had been noticed that the *Ulothrix* became diseased as a result of the parasite, and that the cells lost their chlorophyll and tended to fragment.



Figs. 13-19. *Rhizophyidium globosum*

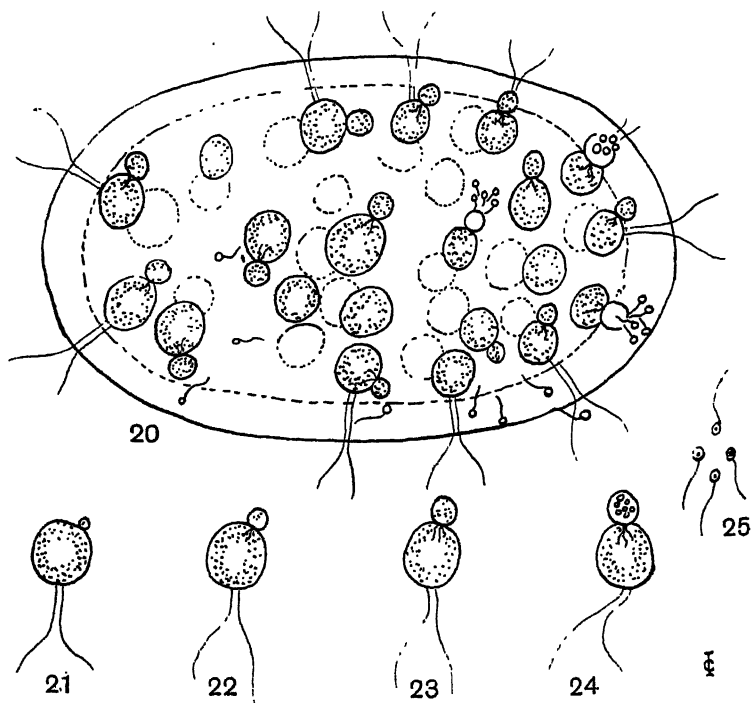
- 13 Infection stage 14 Development of absorption hyphae 15 Growth of zoosporangium. 16 Mature zoosporangium 17 Development of beak to zoosporangium 18. Mature zoosporangium liberating zoospores 19 Zoospores.

This species was first described by A. Braun (3) in 1855 as *Chytridium globosum*, but later was transferred to the genus *Rhizophyidium* by Schroter (13) in 1886. It has been found in a number of aquatic Thallophtyes, including *Cladophora*, *Oedogonium*, *Sphaeroplea*, *Spirogyra*, certain Desmids and Diatoms. In fact it is probably one of the most common species of the genus.

RHIZOPHYDIUM TRANSVERSUM (A. Br.) Fischer

This species was sent to me in 1927 from a pond in Epping Forest. It was growing in the cells of *Eudorina elegans*. At the time this alga was present in very large numbers and the fungus appeared to have attacked a very large proportion of the specimens. The fungus is found producing zoosporangia upon the surface of the individual cells (Fig. 20) of the colony; these zoosporangia are not so spherical as is charac-

teristic of some of the other species. As the zoosporangia grow, the apical end becomes beaked and finally a perforation occurs (Figs. 21-23). Meanwhile the content has become differentiated into a number of zoospores, but so far as could be seen their number is small, not more than about 10 to 15 being produced in each zoosporangium



Figs. 20-25. *Rhizophydium transversum*.

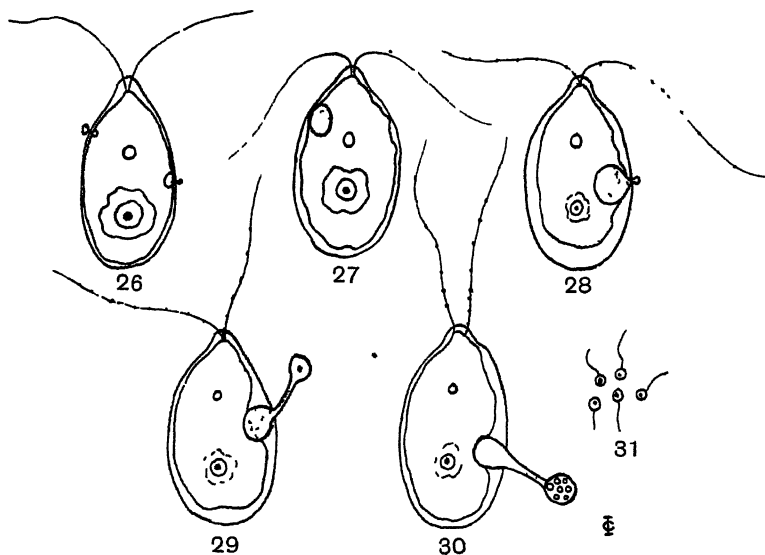
20. *Eudorina elegans* with the fungus in various stages. 21. Single cell of colony with infection stage. 22. Development of zoosporangium. 23. Mature zoosporangium. 24. Zoosporangium with zoospores. 25. Zoospores.

(Fig. 24). On their escape the zoospores possess a single flagellum which is several times as long as the zoospore (Fig. 25). They make their way either to another cell of the colony or force their way through the surrounding mucilage to the exterior. On settling down on a fresh host cell the zoospore loses its flagellum and sends out into the host a tiny process by which food is obtained. It grows in size and the wall surrounding it becomes more clear and finally it develops into a fresh zoosporangium. No evidence of fusion of these zoospores was found.

This species was described by A. Braun (3) in 1855 under the name *Chytridium transversum*. Later Fischer (7) placed it in the genus *Rhizophyidium* owing to the similarity between it and the other species which had been described. So far as the author can discover it has been previously recorded only on *Chlamydomonas* and *Gonium*, but there is nothing in the life-history of this organism to suggest that it is not the same as previously described and it is reasonable to consider that *Eudorina elegans* might quite probably be attacked by a species infecting *Chlamydomonas* or *Gonium*.

RHIZIDIUM APPENDICULATUM Zopf

This species was also collected in a pond in Epping Forest in 1927 and sent to the author. It was found attacking the cells of *Chlamydomonas*, but unfortunately the species could not be determined. The



Figs. 26-31. *Rhizidium appendiculatum*.

26. Early infection. 27. Absorption of fungus into host. 28. Initiation of zoosporangium. 29. Mature zoosporangium. 30. Zoosporangium with zoospores. 31. Zoospores.

host was very common in the pond at the time and most of the cells were infected. The effect on the host was to produce irregularity of the outline of the protoplast and the appearance of beading of the flagella, as well as the assumption of a yellowish-red colour. The life-history could be followed and consisted in the settling down of the

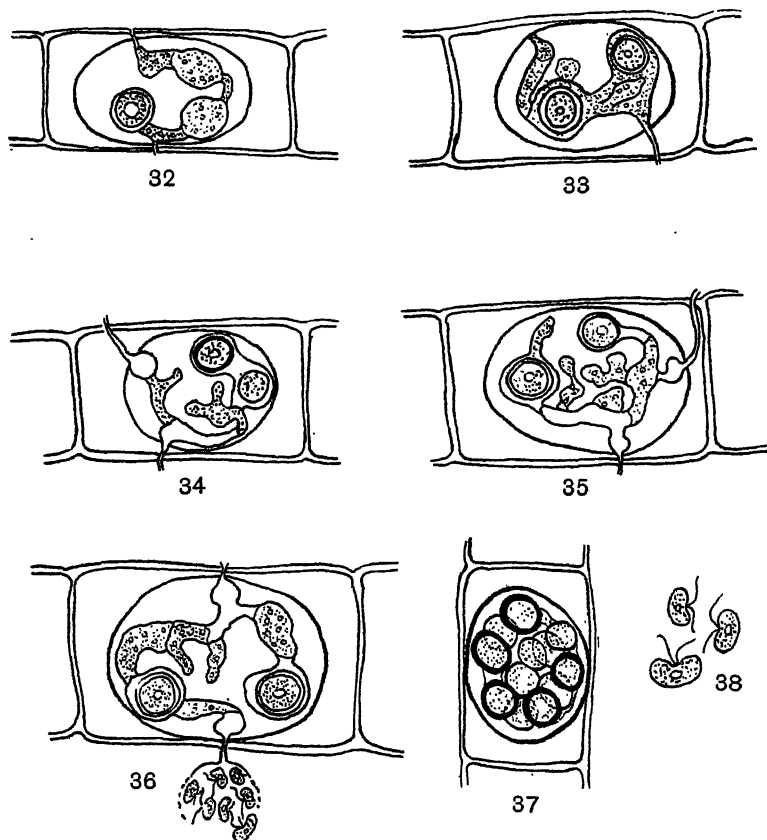
zoospore upon the *Chlamydomonas* cell (Fig. 26), and then slowly penetrating into it, the contents of the zoospore passing through the cell wall by means of a very fine infection hypha. Finally the whole protoplast entered the host (Fig. 27) and began to grow by the absorption of food material from the host. There did not seem to be any particular point of the host where penetration occurred, although infection was not observed at the apical end around the flagella. After a while the fungus sent out a process, or hypha, through the cell wall again (Fig. 28), or more probably the same infecting hypha was used, and the hypha became elongated until it was about twice the length of the body (Fig. 29). The tip became swollen and the whole contents of the organism entered this zoosporangium. After a while the contents of the zoosporangium divided up into zoospores which escaped from the tip of the zoosporangium (Fig. 30). Only a small number of zoospores were liberated, and each was a small spherical structure which possessed a single apical flagellum rather longer than itself (Fig. 31). The zoospores swam actively in the water and infected a fresh *Chlamydomonas* cell. No fusion of zoospores was observed.

This species was described by Zopf (16) in 1884 who found it also in species of *Chlamydomonas* in Germany. In the account given the zoosporangium more frequently is devoid of the marked stalk shown in the illustrations (Figs. 29, 30).

LAGENIDIUM GRACILE Zopf

This species was found in the zygospores of an unidentified species of *Spirogyra*. It was collected in a pond at Dunton Green, Kent, in June 1929 and was extremely common, almost all the filaments were attacked and most of the zygospores were infected. The thallus consists of a ramifying system of hyphae which appears as a mass of coiled tubes lying inside the zygospores. These hyphae are very much branched and it is impossible to follow the course of any one hypha for more than a short distance. When mature the tube becomes segmented into a number of cells, but it was not possible to be certain as to the number of nuclei in each, though it is thought that these are coenocytes and are not related in their formation to the nuclear division. From the tips of the hyphae zoosporangia develop (Figs. 32, 33) which soon form exit tubes which pass through the wall of the zygospore and then through the wall of the cell itself. These exit tubes are very fine and can only be seen with difficulty. Through them the content of the sporangia passes and forms a mass of protoplasm at the exterior. This then gives rise to zoospores (Figs. 36, 38).

In addition to zoosporangia, sex-organs were found; these consist only of oogonia, which develop in enlarged parts of the thallus and are surrounded by a wall. In some zygosporcs the whole content was filled with them and the mycelium could not be made out (Fig. 37).



Figs. 32-38. *Lagenidium gracile*.

32. Young thallus with oospore. 33-35. Thalli with oogonia and sporangia. 36. Thallus showing discharge of zoospores. 37. A zygosporc filled with oogonia and oospores. 38. Zoospores.

Later, without apparently any assistance from an antheridium, they secrete a thick wall (Figs. 34, 35). No stages in their germination were observed, and they appeared as thick-walled bodies with a clear central body and granular surrounding cytoplasm.

Lagenidium gracile was described by Zopf (16) in 1884, and in the same paper he described another species, *L. entophyllum*, which was

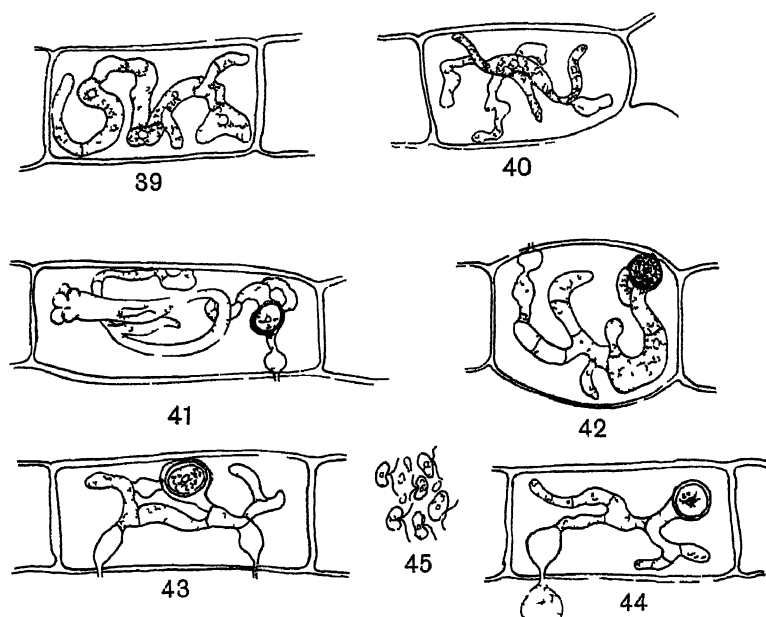
also found in the zygospores of species of *Spirogyra*. These species were also described briefly by de Wildeman (15) in 1895 from Belgium. Later, in 1909 Atkinson (2) gave an account of a further species which he called *L. americanum*, also found in the zygospores of certain species of *Spirogyra*. Doubt has been expressed by some workers as to whether these three are really distinct species, and this by no means helped in the identification of the material I have found. In the opinion of Mr A. W. Bartlett, who very kindly examined the material, it was similar to what Zopf described as *L. gracile*, but Mr Bartlett informed me that he considers that *L. gracile* is very probably only a form of *L. entophyllum*. In Zopf's account of this latter species he considers that a wall is formed around the protoplast after extrusion from the zoosporangium, while Atkinson failed to find such a wall in *L. americanum*. From my own observations I think that a wall is present, although it is on a very thin texture and soon disappears. Atkinson did not find any evidence of sexual reproduction, though in the present material oospores were very common, and almost all the thalli possessed them; no evidence of any antheridium, however, could be seen.

LAGENIDIUM RABENHORSTII Zopf

This species was also present in the same material of *Spirogyra* as the last species, but did not occur so frequently. It was restricted to those filaments which were not in process of conjugation. The thallus ramifies within the host cells in much the same way as the last species, and the hyphae are so much branched that it is impossible to trace their course. The effect upon the host tissue is to disorganise the chloroplast and fragments of it can be seen attached to the fungal mycelium. These hyphae are composed of granular cytoplasm in which are embedded numerous refractive bodies (Figs. 39, 40). The formation of zoosporangia follows very much the same course as has already been described. After their formation exit tubes are developed which are very fine hyphae passing out through the cell wall (Figs. 41, 42). As a general rule the exit tubes in this material were very short. The content of the zoosporangium becomes more densely filled with cytoplasm and then passes through the exit tube to the exterior. Here it forms a small papilla (Fig. 44) which appears to be enclosed in a thin wall, and while still enclosed divides up into a number of zoospores. These zoospores are kidney-shaped and possess two laterally placed short flagella (Fig. 45).

Sexual reproduction, by the development of both antheridia and

oogonia, was observed (Fig. 42) and in some cells mature oospores were seen. No stages in the development of these oospores (Fig. 43) could be found. It seems likely that they may be resting structures and do not germinate till after they have been liberated by the breakdown of the host tissue.



Figs. 39-45. *Lagenidium Rabenhorstii*.

- 39 Thallus in host cell. 40. Young thallus in host cell. 41. Thallus with oospore and sporangium. 42. Thallus showing antheridium and oogonium. 43. Thallus with two discharged sporangia. 44. Sporangium just after discharging contents. 45 Zoospores

Lagenidium Rabenhorstii was described by Zopf(10) and it has also been examined further by Atkinson(2) and others(1,15), so that our knowledge of this species is fairly complete. The chief interest in the material I have found lies in the fact that the organism has not been previously recorded in this country despite the fact that it has been found both in Europe and America.

I wish to thank Mr A. W. Bartlett for his assistance in examining some of the species described and for looking through the manuscripts.

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STUDIES IN FLORAL MORPHOLOGY

IV. ON THE HYPECOIDEAE, WITH SPECIAL REFERENCE TO THE ANDROECIUM¹

By AGNES ARBER

(With 12 figures in the text)

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I. INTRODUCTION

ACCORDING to Hutchinson (10) the Fumariaceae should be regarded as a family distinct from the Papaveraceae and including two sub-families. In the preceding paper of this series (4) I have considered the flower structure of one sub-family, the Fumarioideae; the present instalment deals with the other sub-family—the Hypecoideae. This group consists of three genera—*Chiazospermum* (2 species), *Hypecoum* (14 species) and *Pteridophyllum* (1 species); but *Chiazospermum* is sometimes included under *Hypecoum*. The present work is based

¹ For the titles of the preceding papers of this series, which forms part of the work carried out with the aid of a grant from the Dixon Fund of the University of London, see References (2)–(4), p. 173.

on fresh specimens of three species of *Hypecoum* and dried flowers of one species of *Chiazospermum*. I have not had an opportunity of examining the monotypic *Pteridophyllum*¹. For material and other help I am indebted to the Director of the Royal Botanic Gardens, Kew, to the Director and the Superintendent of the Botanic Garden, Cambridge, and to Mr F. H. Wright.

In the following pages the flower structure of the species available is first described, and then the bearing of these observations is discussed.

2. DESCRIPTION

(i) *Hypecoum leptocarpum* Hook. f. et Thoms.

Fig. 1, A₁, p. 147, shows part of an inflorescence of *H. leptocarpum*. This inflorescence is dichasial; each flower terminates a shoot, and two younger flowers may be borne in the axils of its bracteoles. The flowers consist of few parts—two sepals, four petals, four stamens and two carpels. The nature of the vascular system can be understood from the series of sections through a peduncle and flower drawn in B₁–B₁₉, Figs. 1–6). In Fig. 1, B₁, the peduncle is cut through below its bracteoles; it shows eight bundles, which, for convenience of description, are labelled A–H. Fig. 1, B₂ and B₃, and Fig. 2, B₄ and B₅, p. 148, show the origin of the bracteoles and of the pedicels which are borne in their axils. In the main the bracteoles receive their median bundles from C and G, while minor bundles arise from B and D, and from F and H. The cylinders for the lateral pedicels are formed from H and F, and from B and D; while A and E are responsible for the vascular system of the terminal flower. These two bundles (A and E) each divide into three, so that there come to be six bundles in the pedicel between the bracteoles and the flower (Fig. 2, B₄). In B₆ and higher diagrams, the pedicel of the terminal flower is shown alone. The six bundles derived from A and E have undergone further divisions by the time B₆ is reached, and in order to make their description easier, fresh lettering is introduced at this point. The four bundles, α_1 – α_4 (two of which are derived from A and two from E) have become the most prominent features of the section in B₇. They are of special importance in the flower, as they provide the greater part of the phloem for the stamens, and all the xylem and phloem for the gynaecium. It will be most convenient to follow the history of the gynaecium strands now, and to deal later with the exterior whorls. In B₇ it will be noticed that the xylem of the α bundles is curiously

¹ See Note, p. 173.

HYPECOUM LEPTOCARPUM

Hook. f. et Thoms.

A₃-A₅ sections of an ultimate branchlet of a dichasium. The opposite bracteole was given off at a lower level.

young flower of second order

inner petals

sepal

flower of 3rd order

bracteole of second order

bracteole of first order

A₁

A₃ bundle which supplies axillant leaf & bud (= bracteole & axillary pedicel)

A₂

inner face of inner petal showing median lobe

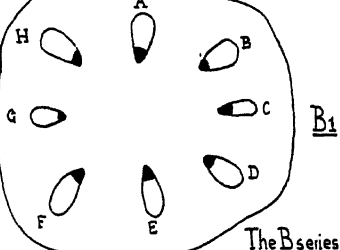
fruit of flower of the first order

A₄

branch of bud bundle forming a lateral for the axillant leaf

A₅

bud (pedicel) bundles median bundle of axillant leaf (bracteole)

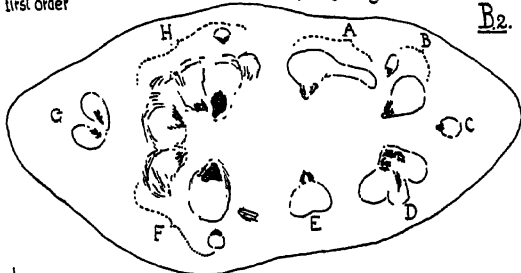


B₁

The B series

in this & succeeding figures, is from a dichasium such as X in A₁, but younger

B₂



cylinder for lateral pedicel, formed from H & F

cylinder for lateral pedicel formed from B & D with a small contribution from A

base of bracteole supplied by G & by a branch from F & from H

strands for terminal flr

base of bracteole supplied by C & a branch from B & from D

B₃

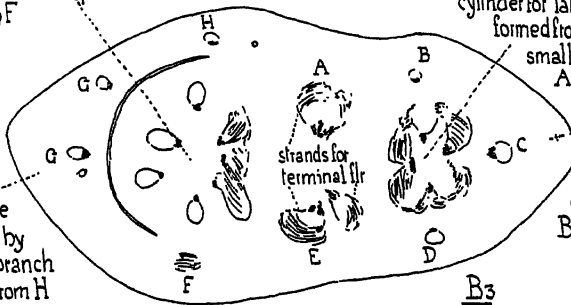


Fig. 1. *Hypocoum leptocarpum* Hook. f. et Thoms. (Cambridge Botanic Garden.) A₁, part of a minor dichasium (enlarged). A₂, inner face of inner petal (enlarged). A₃-A₅, sections (× 56) from a transverse series from below upwards through a very young axis from an inflorescence bud, to show the vascular relation of an axillant leaf (bract) to its bud (pedicel). B₁-B₃, sections (× 56) from a transverse series through a peduncle, pedicel and flower, which is continued in B₄-B₁₀, Figs. 2-6.

HYPECOUM LEPTOCARPUM

Hook. f. et Thoms.

(continuation of series in preceding figure)

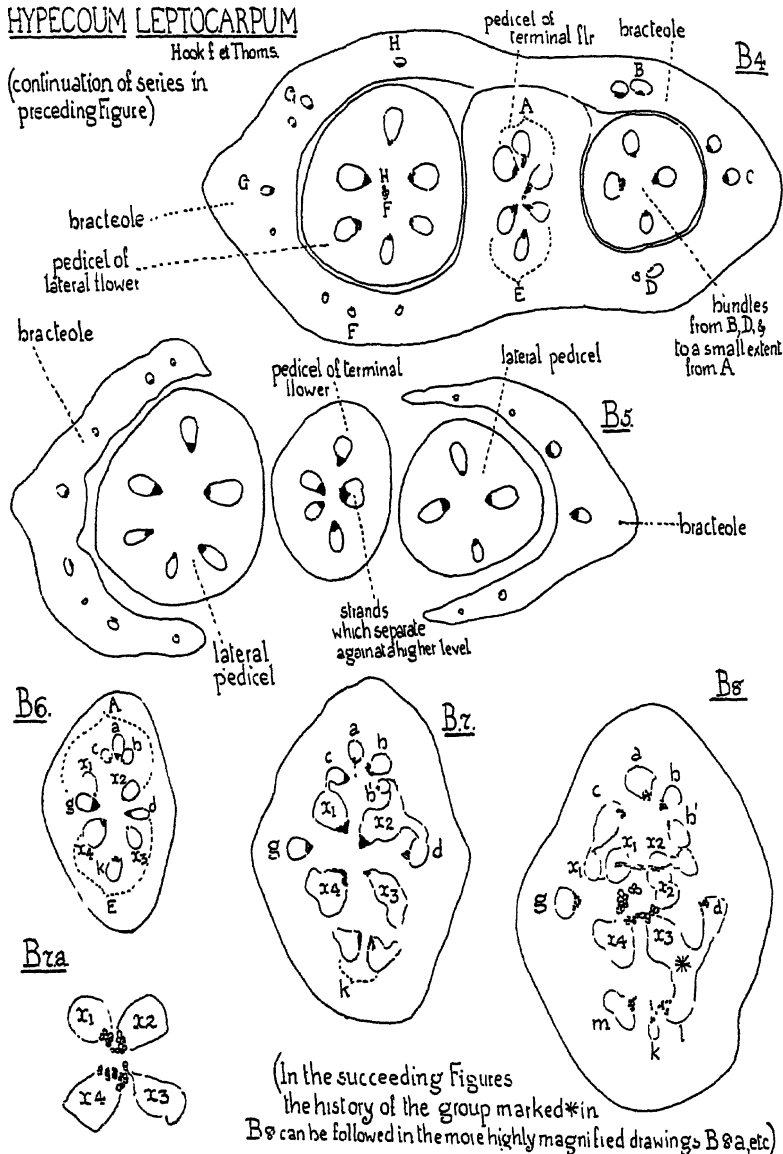


Fig. 2. *Hypecoum leptocarpum* Hook. f. et Thoms. B₄-B₈, continuation of the series begun in Fig. 1 ($\times 56$). In B₆-B₈ the bracteoles with their lateral pedicels are omitted. B₇a, the four bundles, x₁-x₄, in B₇, at a slightly higher level; the lignified elements are indicated individually. In B₈ a fresh system of lettering is introduced to enable the bundles to be followed into the flower.

unsymmetrical. This point is clearer in B_7a (a little above B_7), in which the actual elements of the xylem are indicated; x_3 has two elements, while x_4 has about a dozen. The four xylems approach one another, so as almost to form a ring, and their elements then become redistributed into four groups with a north, south, east and west orientation, replacing the original oblique orientation (Fig. 3, B_9a , p. 150). The details of this process are difficult to follow, and the mode of division and anastomosis of the phloems of x_1 – x_4 is of still greater complexity¹. As regards their relation to the gynaeceum, it may be said—broadly—that, by a process of bifurcation, followed by fusion of the branches two by two, they produce four phloem masses ($x_1 + x_2$), ($x_2 + x_3$), ($x_3 + x_4$), and ($x_4 + x_1$), which lie in the north, east, south and west planes, and serve the midribs of the carpels and their fused marginal strands.

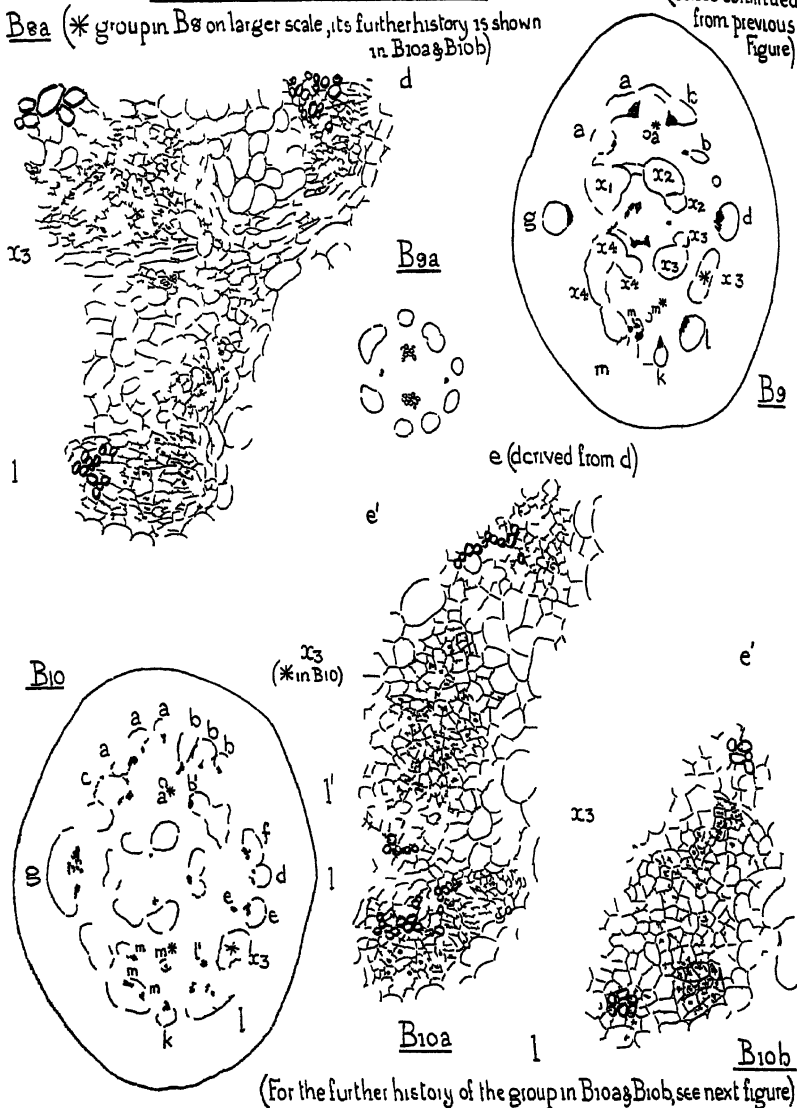
Leaving the gynaeceum bundles and turning to the outer part of the receptacle system, we see in Fig. 3, B_9 and B_{10} , evidence of activity in the a, b, c group to the north and the k, l, m group to the south. A curious feature is the presence of two delicate inner strands, a^* and m^* , each consisting of one xylem element only, and derived respectively from the protoxylems of a and of m' . By the time B_{10} is reached, a^* has lost its lignification, while at a higher level m^* fuses with one of the branches of l to form the midrib of an inner petal. The bundles k and a provide the midribs of the sepals. To the south, the midrib of the petal placed immediately within the sepal is formed by contributions from l and m , while the wing bundles, l and m , each subdivide to give a sepal lateral and a petal lateral. In the l group to the south-east (and in the a group to the north-west) the division produces three bundles instead of two, but two of the three fuse again to form a single petal lateral. As there is only one pair of sepals (north and south) the vascular system is simplified in the east and west in correlation with the absence of calyx members in this plane. In B_{10} the bundle g to the west is beginning to divide into three, while d , to the east, has already branched into d, e and f . In Fig. 4, B_{11} , the median bundles, d and g , and their laterals (e and f, h and i) are ready for the outer petals.

It now remains to trace the origin of the bundles for the androeceum—the part of the flower which specially concerns us. Returning to the level of Fig. 2, B_7 , p. 148, we see that the phloems of the x bundles are forming connections with other strands. The strand x_2

¹ In this description the details are somewhat simplified; see the section on the phloem nexus, p. 165.

HYPERCOUM LEPTOCARPUM Hook f et Thoms (Series continued)

B_{ea} (* group in B₈ on larger scale, its further history is shown in B_{10a} & B_{10b})



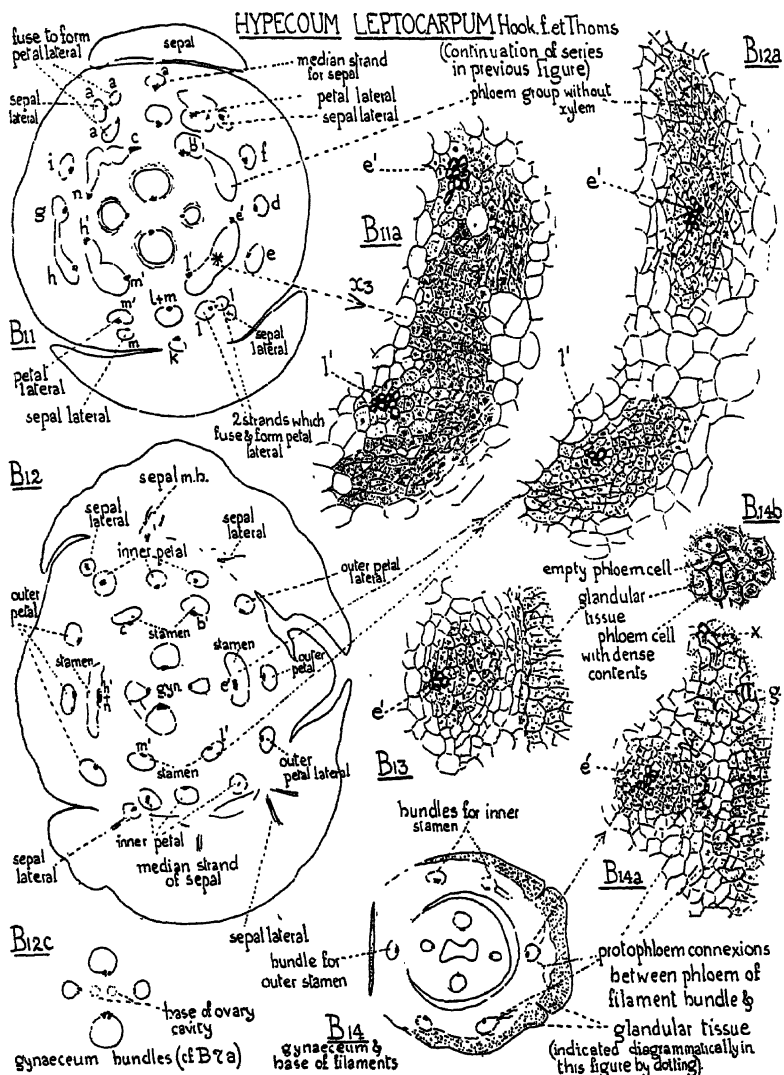


Fig. 4. *Hypecoum leptocarpum* Hook. f. et Thoms. Continuation of the series in Figs. 1-3. B₁₁, B₁₃, B_{12c} and B₁₄ (× 56). B_{11a}, B_{12a}, B₁₃ and B_{14a} (× 232). B_{14b} (× 382). In B₁₃ and B_{12c} the gynaeceum bundles are somewhat simplified. B_{11a} and B_{12a} form a continuation of the series illustrating the development of the south-east stamen band, which is begun in Fig. 3. B_{3a}, B_{10a} and B_{10b}. B₁₃, B_{14a} and B_{14b} continue the history of e', and the xylemless phloem group to the north which becomes associated with it to form the bundle of the outer stamen on the east. B₁₄, slightly oblique, shows the gynaeceum and stamens only, in the region below the freedom of these members. The dotting in this diagram is used to indicate the glandular tissue. B_{14a} shows the bundle and part of the glandular tissue g of the east stamen in B₁₄, and the protophloem strands connecting with the gland tissue. B_{14b} shows the region X in B_{14a}, on a larger scale.

is united in B_7 with the phloem of b' (a branch of b) on one side, and with that of d on the other. In B_8 the phloem of x_1 is connected with c , and at a level a little above B_8 , that of x_4 forms a junction with that of m . It will be sufficient to follow in detail the further history of one only of these groups; we may choose x_3 , which in B_8 is connected both with d and with l (one of the triad k, l, m , which has arisen from the single bundle k in B_6). This group is distinguished by an asterisk; it is shown more highly magnified in Fig. 3, B_8a , p. 150. In B_9 , the phloem mass marked * has become detached and lies between d and l . In $B_{10} a$, d and l are each contributing a xylem branch on the side towards the phloem strand, x_3 . (One of these, l' , is a branch of l , while the other, e' , is a branch of e , which was itself given off from d .) In $B_{10} b$ these strands are approaching the phloem band; neither of them is accompanied by phloem of its own. In Fig. 4, $B_{11}a$, p. 151, the margins of the phloem band have become closely associated with e' and l' , so that the result is a broad phloem band connecting two xylem groups. It will be seen in B_{11} that the phloem band, whose history we have traced, is one of four such bands, symmetrically placed, truncating—as it were—the corners of a rectangle enclosing the gynaeceum strands. The history of the other groups is essentially the same as that of the group described. The phloem mass of one is derived from $(x_1 + c)$ and those of the other two—in the main—from x_2 and x_4 respectively. The lettering indicates the sources of their xylems. But the previous connections, which the phloems of the x bundles have formed with those of other strands, add a further complexity which it is impossible to express in the lettering. In the individual flower illustrated, there are two aberrant features. The xylem group, n , at the south margin of the north-west vascular band, arises *de novo*, a little below the level of B_{11} , instead of being a branch of i , as one would expect by analogy with the other strands; and f fails to supply a xylem group for the south extremity of the north-east phloem band, so that this band is unsymmetrical in having xylem at one end only. But these individual peculiarities do not obscure the general scheme, which is shown diagrammatically in Fig. 7, 1, p. 156.

The next stage above B_{11} is that each of the four phloem bands divides into two radially, so that each of the eight¹ xylem groups has its own phloem strand (Fig. 7, 2, p. 156). Almost simultaneously with this division, the east and west half-bands fuse in pairs. The

¹ One of the xylem groups was absent, as already explained, in this individual flower, but in another flower the full number, eight, were present.

result is that each of the stamens to east and west is supplied by one bundle, single in appearance but double in origin, having arisen by the fusion of adjacent halves of two initially independent vascular bands. The stamens to the north and south each, correspondingly, receive two half-bands, but no fusion takes place, so that the filaments remain two-bundled (Fig. 7, 3). Since the east stamen in the

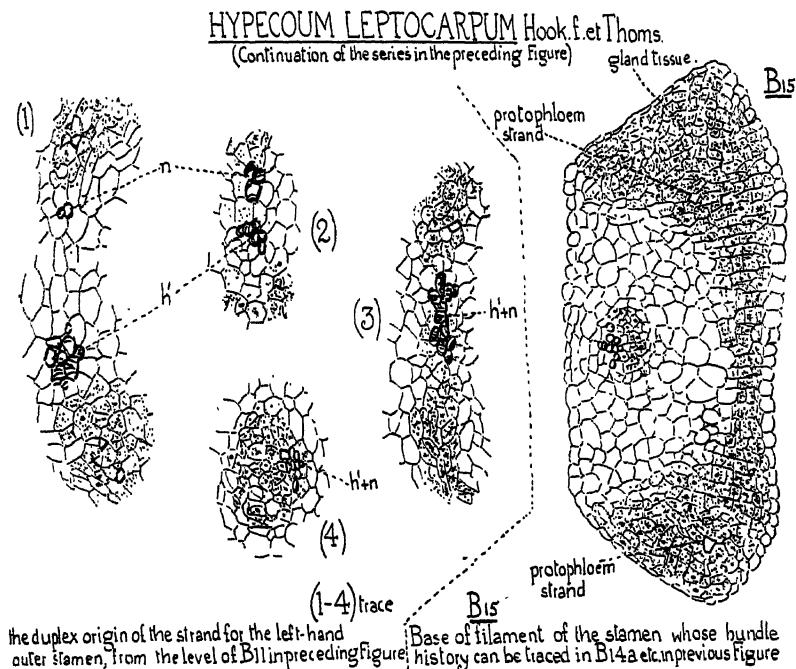


Fig. 5. *Hypecoum leptocarpum* Hook. f. et Thoms. B₁₁a-B₁₅ (× 232), continuation of the series in the previous figures. B₁₅ is the conclusion of the series formed by Fig. 3, B₈a, B₁₀a, B₁₀b and Fig. 4, B₁₁a, B₁₂a, B₁₃, B₁₄a. B₁₅ shows the right-hand outer stamen at a level at which the filament has become free; in the preceding figures of this series the vascular tissue was still in the receptacle. (1)-(4), series showing how the single bundle of the left-hand outer stamen originates from two strands in the receptacle. (1) is a few sections above B₁₁, Fig. 4.

flower illustrated lacks one of its xylem groups, the process of fusion, which has been accomplished in Fig. 4, B₁₂a, is not typical, for the phloem accompanying *e'* merely fuses with the xylemless phloem mass to the north. So the actual process of the derivation of one bundle from two is better followed in the bundle for the west stamen, which is drawn in Fig. 5, (1)-(4).

It will be seen in this series that the xylems first approach one another and fuse, while the union of the phloems occurs later. Returning to the east stamen, we may now follow the bundle into the filament. Fig. 4, B_{13} , shows the strand at the extreme base, before the filament has begun to detach itself from the receptacle. The cells to the right represent the first trace of the glandular tissue, which is a feature of the filament base. In B_{14} , which is a little higher, the eastern stamen (which we are following) has more glandular tissue, and this tissue, which is indicated in the diagram by dotting, is penetrated by branchlets of an extremely slight and delicate kind, from the phloem of the vascular bundle. These connections, which can be seen in greater detail in $B_{14}a$ and $B_{14}b$, apparently consist of fragile protophloem fibrils, which may be assumed to form a conducting system for the gland. The fibrils include two types of element, both of which have walls which stain more sharply than those of the glandular tissue. In order to distinguish these walls, they are represented in $B_{14}b$ as thicker than they actually are; their difference from the walls of the glandular tissue lies, in fact, rather in staining capacity than in thickness. One of the two types of phloem element is empty, while the other has dense contents. These contents are somewhat different in appearance from those of the gland cells; the nuclei are less prominent, and the cytoplasm is denser. Like the gland cells, some of the phloem elements show a tendency to a radial arrangement. In Fig. 5, B_{15} , in which the whole filament is shown, the protophloem fibrils have almost disappeared. Some empty protophloem elements can still be traced in either flank, but the phloem cells with contents—if they are present at this level—cannot be distinguished from the surrounding glandular tissue, which now forms a continuous zone occupying the dorsal and marginal region of the filament section. Still higher, in Fig. 6, B_{16} , the glandular tissue has become confined to the margins of the filament. The fibrils do not reach to this level in the eastern stamen; but as the section is slightly oblique—cutting the flower at a lower level in the west than in the east—fibrils can be detected in the glandular masses of the stamens to the west. In B_{19} the flower which we have been studying is cut through its anthers and styles, and it is seen that the two-bundled condition of the inner stamen filaments persists into the anther. Indeed the two bundles retain their identity above the level of the pollen-sacs ($B_{19}a$), but it should be noticed that the anther is not bicleft.

I have cut serial sections of nine flowers, and in eight of them I

HYPECOUM LEPTOCARPUM Hook f. et Thoms
(Continuation of series in previous figures)

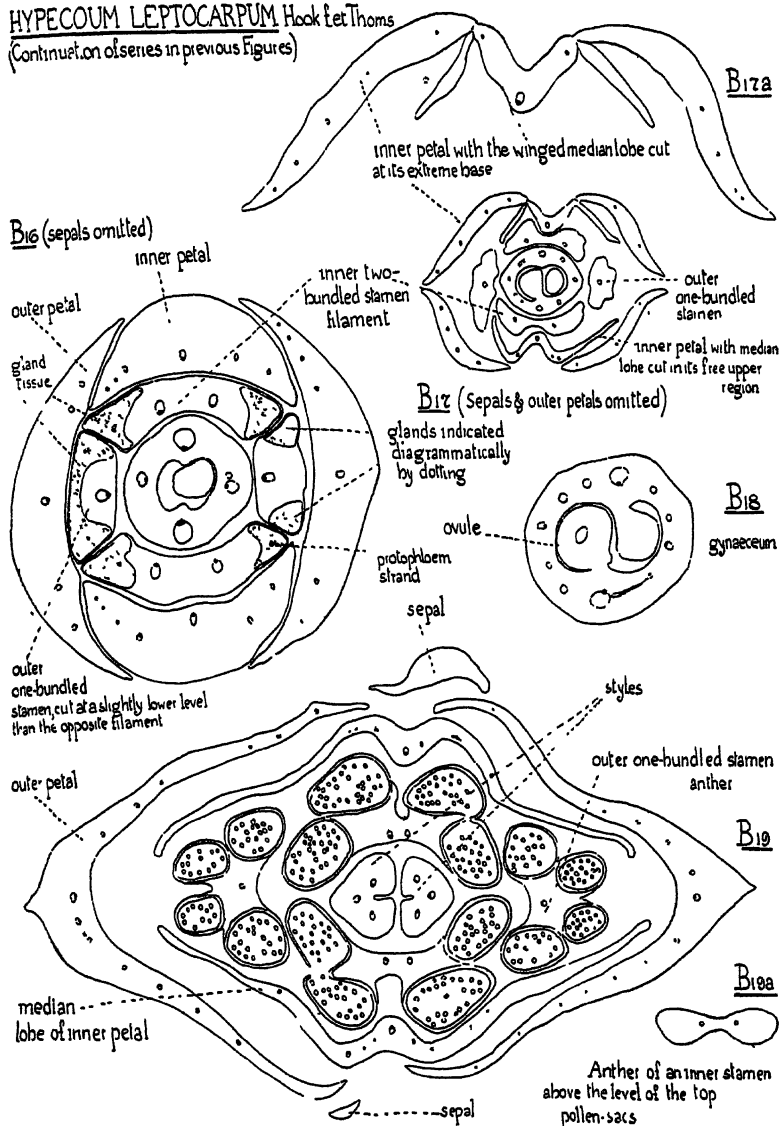


Fig. 6. *Hypecoum leptocarpum* Hook. f. et Thoms. Continuation of the series in previous figures. B₁₈, B_{17a}, B₁₈, B₁₉, B_{19a} ($\times 56$); B₁₇ ($\times 28$). B₁₈, sepals omitted. B₁₇, sepals and outer petals omitted. B_{17a}, one of the inner petals of B₁₇, showing the ventral lobes at their extreme base. B₁₈, gynaeceum only. B₁₉, whole flower at level of anthers and styles. B_{19a}, one of the two-bundled inner stamens from another flower, near the extreme tip above the pollen-sacs, to show that even at this level the filament is not bifid.

have found the scheme of stamen bundles figured in B_{19} —that is to say, one bundle to each of the outer stamens, and two bundles to each of the two members of the inner whorl. But the ninth flower (illustrated in Fig. 8, D, p. 157) diverged from this type in having a single bundle in one of the two inner stamens. Within the filament, however, this bundle gave off an unligified branch, which had died out below the level of the diagram.

Apart from the androecium, an unusual feature of the flower is the curious trilobing of the two inner members of the corolla. One of these inner petals is shown in Fig. 1, A_2 , p. 147, in surface view, and in section (at the base of the median lobe) in Fig. 6, B_{17} and B_{17a} , p. 155, while the upper part of the median lobe, above the lateral lobes, is cut in B_{19} .

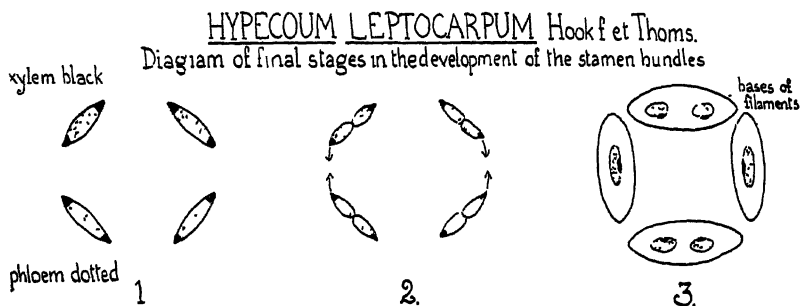


Fig 7. *Hypecoum leptocarpum* Hook. f. et Thoms. Diagrams showing the penultimate and ultimate stages in the arrangement of the vascular tissue for the stamens.

In describing the gynaecium bundles in the B series, I have mentioned the extreme complexity of the phloem. This is illustrated from another flower in Fig. 8, C_1 and C_2 , p. 157, which are sections of the receptacle at levels near the insertion of the sepals. This phloem nexus will be considered later (p. 165).

The general morphology of the gynaecium needs no special description. Fig. 6, B_{18} , shows the ovary, and B_{19} , the base of the stylar branches. The structure is bicarpellary, with the ovules borne on the fused margins of the carpels; the carpels become free from one another in the stylar region.

as a series to the gynoecium
 in the center of the
 flower

HYPECOUM LEPTOCARPUM

Hook & Thoms

C



C2b left hand bundle at a slightly higher level than C2 to show phloem islets more magnified

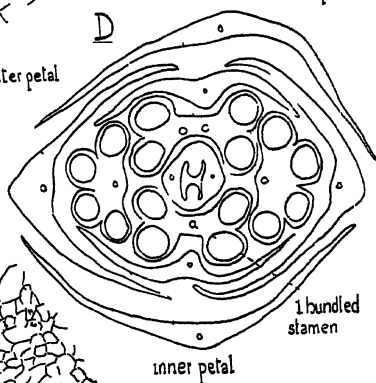


2-bundled stamen

sepal

D

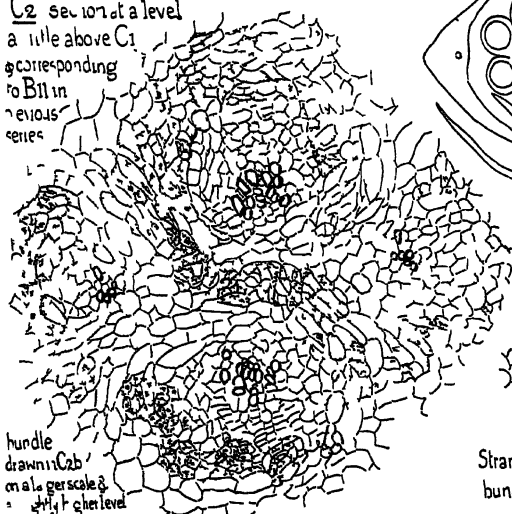
outer petal



1-bundled stamen

inner petal

C2 section at a level a little above C1 corresponding to B1 in previous series



hurdle drawn in C2b on a larger scale

C2a



Strand of phloem between the two main bundles in C2, on a larger scale

Fig 8 *Hypocoum leptocarpum* Hook & Thoms Transverse sections from another flower from that drawn in Figs 1-6 C₁ and C₂ (x 232) C_{2a} and C_{2b} (x 382) C₁ section of the central region of the receptacle at a level a little below Fig 4 B₁₁ C₂ the same region at a level corresponding to Fig 4 B₁₁ C_{2a} the phloem strand which crosses the receptacle between the placental bundles on a larger scale D transverse section of another flower (very young) in which only one filament is two bundled The single bundle in the other filament gave off a non lignified branch which disappeared below the level of this section (x 56)

HYPECOUM PROCUMBENS L

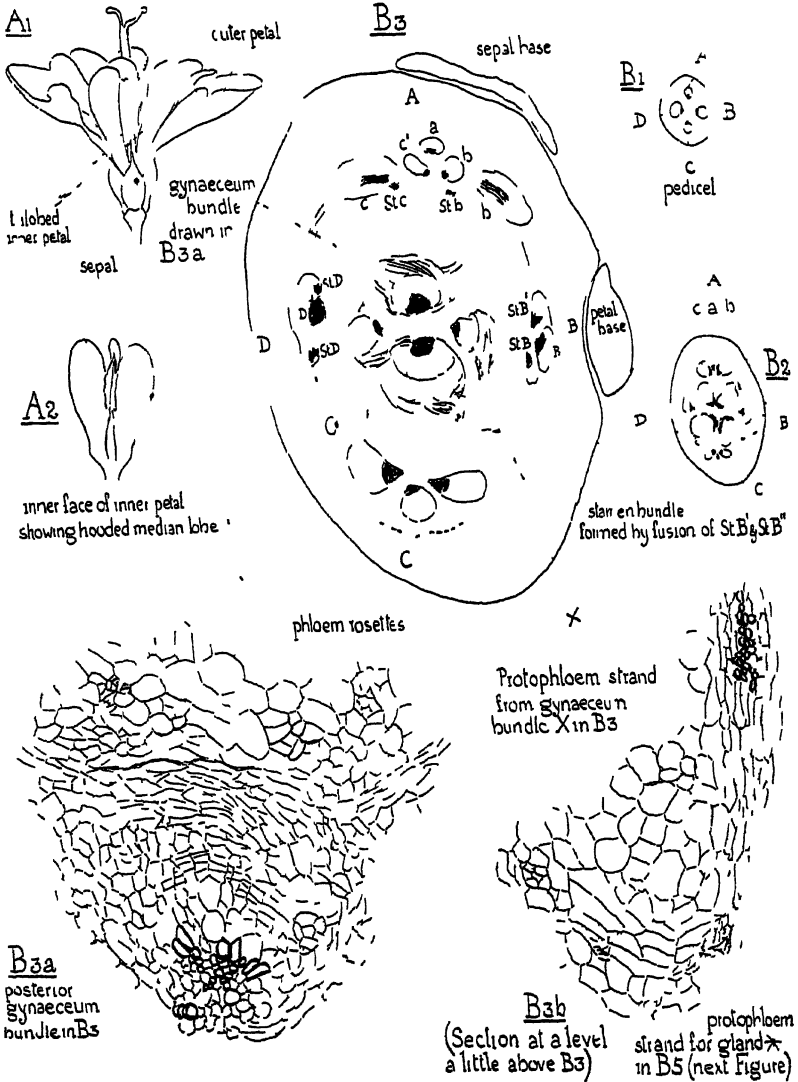


Fig. 9 *Hesperocoum procumbens* L. (Royal Botanic Gardens, Kew) A₁, flower, A₂ inner petal (both enlarged) B₁–B₃ (and B₄ and B₅, Fig 10), sections from a transverse series through a flower B₁, pedicel B₂, receptacle below base of calyx (× 17) B₃, at the level at which the bases of sepals and petals are just coming into view (× 56) B₃a, gynaecium bundle to the north in B₃ to show the horizontal and vertical wefts of protofloem associated with the phloem of the gynaecium bundles (× 232) B₃b, connection between a protofloem strand arising from bundle X in B₃ and the gland strand marked * in Fig 10, B₅ (× 232)

(ii) *Hypocoum procumbens* L.

It is not necessary to describe *Hypocoum procumbens* so fully as *H. leptocarpum*, since in many points the flower structure of the two species is alike. Fig. 9, A₁ and A₂, p. 158, shows the general appearance of the flower and of an inner petal. Fig. 9, B₁–B₃, and Fig. 10, B₄ and B₅, p. 160, illustrate some of the sections from a series through a flower-bud. In B₂ we have a stage corresponding roughly to that drawn for *H. leptocarpum* in Fig. 2, B₇, p. 148. At this level we see an early phase in the formation of wefts of delicate phloem elements, which take a more or less horizontal course between the bundles. In Fig. 9, B₃ (which is on a larger scale), a level is reached at which many of the strands for the different members of the flower are already individualised, and the four gynaeceum bundles have taken up their definitive position. The irregular branching of the proto-phloem, noted in B₂, is particularly conspicuous, many of the strands being vertical (B₃a). The bundle A to the north of the section in B₁, has divided into three strands, *a*, *b*, *c*, in B₂. The middle strand, *a*, forms the median bundle of the sepal. The next stage, shown in B₃, is the further division of each of the wing bundles, *b* and *c*, into three strands—*c'*, *c*, and *Stc''*; *b'*, *b*, *Stb''*. At a higher level *c'* and *b'* fuse to form a petal bundle, while *Stc''* and *Stb''* fuse similarly to form a stamen bundle (see also Fig. 10, C, p. 160). A corresponding process occurs in the bundle to the south, C, but, owing to a slight obliquity of the section, this diagram shows only the triad division of C; the further divisions can be seen in Fig. 10, B₄. The two other main bundles, B and D, have a simpler history, for they divide into three bundles only—B, *StB'*, *StB''*; D, *StD'*, *StD''*. In each group the median bundle supplies the petal, while the two wing bundles fuse to form a stamen strand. For the right-hand stamen, this fusion has taken place in Fig. 10, B₄. In Fig. 10, B₅, the parts of the flower have all separated from one another. The four filaments have each become one-bundled by fusion of paired strands in the receptacle. It will be noticed that though the origin of the xylem strands for the androeceum of *H. procumbens* essentially recalls the corresponding process in *H. leptocarpum*, there is no appearance, as in *H. procumbens*, of the four "phloem bands" which give such a special character to the androeceum system of *H. leptocarpum* (Fig. 4, B₁₁, p. 151, and Fig. 7, 1, p. 156). I examined serial sections of eleven flowers of *H. procumbens* and in eight of them I found all four filaments one-bundled, as in Fig. 10, B₅. But in one of the remaining flowers, I

HYPECOUM PROCUMBENS L.

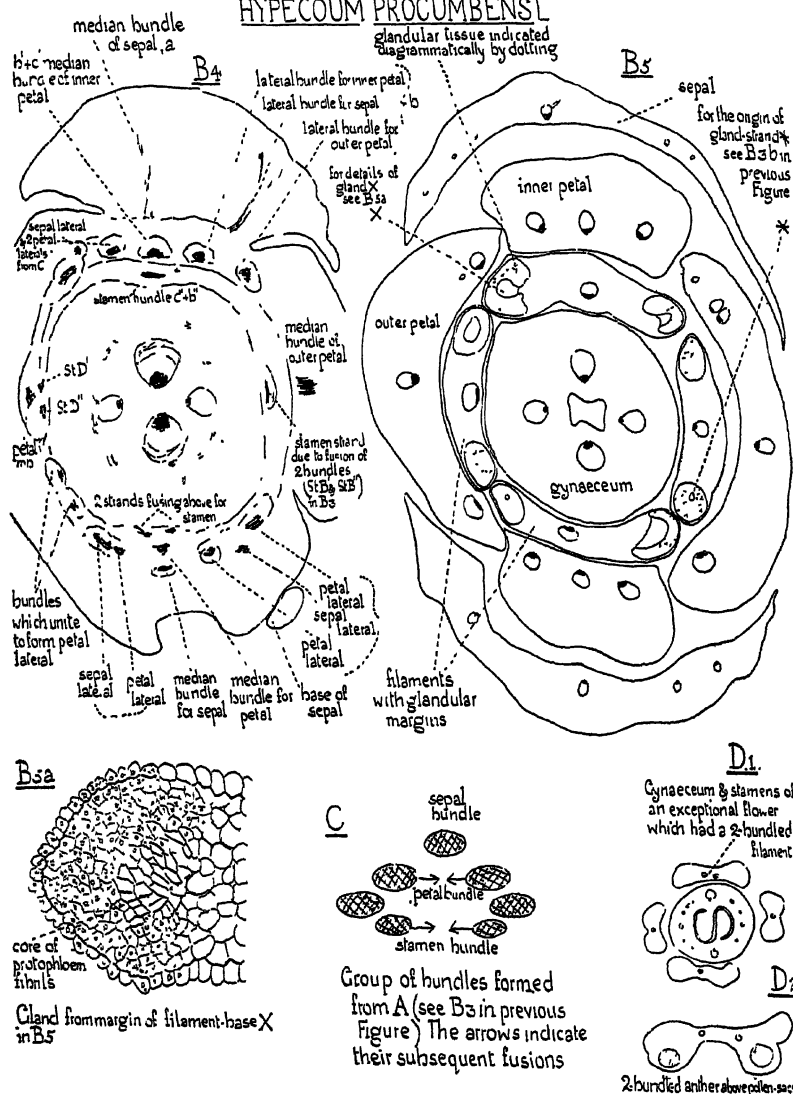


Fig. 10. *Hypecoum procumbens* L. B₄ and B₅, continuation of the series (× 56) in Fig. 9. B₅ is near the base of the filaments. B₅a, glandular filament-margin marked X in B₅ (× 232). C, diagram to illustrate the duplex origin of the median strand for the inner stamen which is opposite to it. All the strands shown originate by branching of one bundle. D₁, section from a transverse series (, 28) through one of the two flowers (out of eleven examined) which had two bundles in one filament. D₂, the two-bundled stamen near the extreme tip above the anther-cavities (× 56). The shaded areas are the fibrous layers of two of the sacs cut tangentially.

found one filament with an indication of doubleness in the xylem, while the other two flowers each had one of the inner stamens two-bundled (Fig. 10, D). These two aberrant flowers were laterals below the normal terminal flower, and the two-bundled filament belonged in each case to a posterior inner stamen. Returning to Fig. 10, B_5 and B_5a , we see that in the marginal region of each filament base the tissue is apparently glandular. Both epidermis and mesophyll have denser cell contents and more conspicuous nuclei than the rest of the filament. But there is a central region of elements with cell walls which are more sharply defined and angular than those of the surrounding tissue; these elements generally seem to be empty, or to have non-stainable contents. This core may be interpreted as a conducting strand of protophloem fibrils; if phloem cells with contents are present, they are indistinguishable from the glandular tissue. The gland strands are extremely delicate and elusive, and it is difficult to trace them to their sources, but in the example drawn in Fig. 9, B_3b (and, I believe, in others also) I have traced a connection between these fibrils in the stamen gland and a strand arising from one of the gynaeceum bundles and belonging to the phloem nexus which has been already mentioned.

(iii) *Hypocoum pendulum* L.

I have cut sections of four flowers only of *H. pendulum*; in each of these the stamens were all one-bundled (Fig. 11, A, p. 162). The history of the bundle for the north stamen can be traced in B_1 – B_4 . B_4 shows the paired bundles destined for this stamen, while they are still in the receptacle. In B_3 these bundles have fused, and the distal part of the phloem of each has separated off to form a gland strand which in B_2 is becoming associated with the gland tissue. In B_1 the filament itself is reached. But the gland strands do not always—as in this example—arise from the stamen-phloem. In other cases these strands can be traced to the delicate nexus of protophloem which arises in the receptacle just below the detachment of the sepals. This nexus originates from the phloem of the four bundles corresponding to those in *H. leptocarpum* which are labelled κ_1 – κ_4 in Fig. 2, B_6 – B_8 , p. 148.

(iv) *Chiazospermum erectum* Bernh. (*Hypocoum erectum* L.)

In microtome sections of herbarium material of *Chiazospermum erectum*, collected in August, 1872, in Transbaikalia, Siberia, I found that the outer stamens were one-bundled and the inner stamens

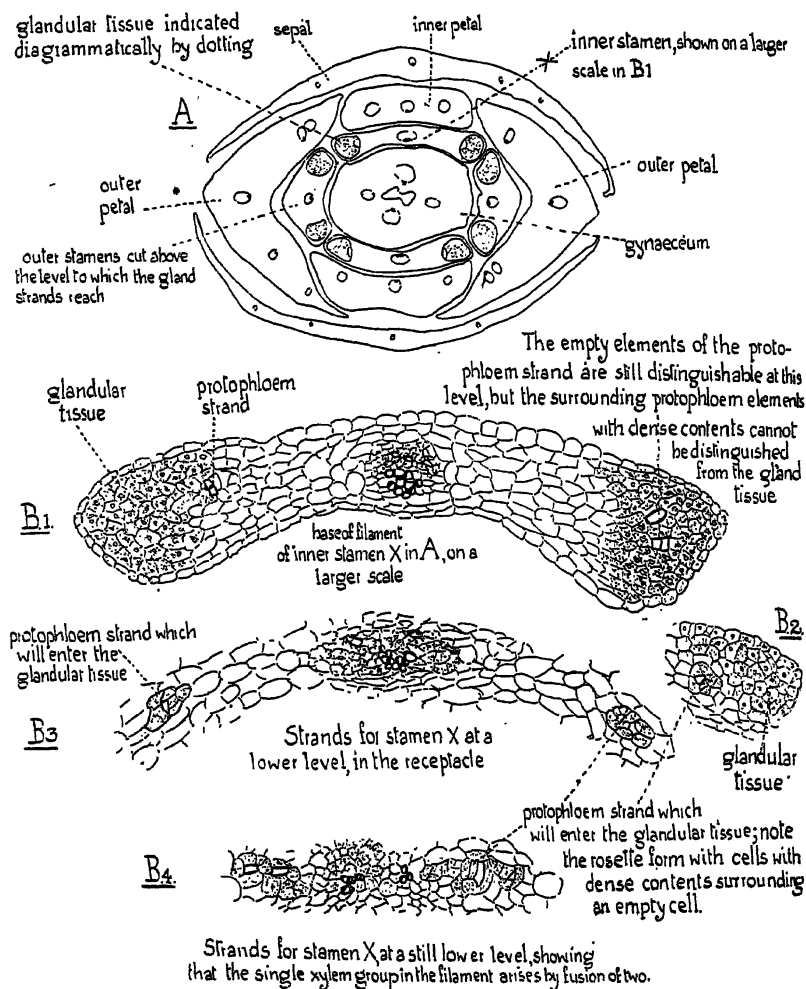
HYPECOUM PENDULUM L

Fig. 11. *Hypecoum pendulum* L. (Cambridge Botanic Garden.) A, transverse section of a flower ($\times 56$) passing through the base of the filaments and gynaecium. B₁-B₄, sections from a series from above downwards through the base of the filament of the north stamen in A, and the strands in the receptacle destined for it ($\times 232$). B₁, at the level of A. In B₄, which represents the margin only of the stamen, the detachment of the stamen base from the receptacle has begun, but is not completed. B₂, lower than B₁ and B₂, below the detachment of the stamen base from the receptacle; B₄, at a lower level, showing that the single xylem group of the filament originates through the fusion of two xylem groups.

two-bundled. In two flowers I traced the origin of the single xylem groups for the one-bundled filaments, and found that these groups each arose from two strands.

3. DISCUSSION

(i) *The inner petals*

The inner petals of *Hypecoum* are trilobed, with a median lobe which may be hooded or spatulate: it is shown for *H. leptocarpum* in Fig. 1, A₂, p. 147, and for *H. procumbens* in Fig. 9, A₂, p. 158. Great stress has been laid on this feature by Eichler(7), who describes the middle lobe as "in Gestalt einer sterilen Anthere." It is undoubtedly possible to interpret corollas in general as equivalent to sterile androecia, but Eichler goes much beyond this when he definitely homologises the middle lobe of the *Hypecoum* petal with an anther. Hildebrand(9) has pointed out that the pollen is shed before the flower opens, and is caught within the middle lobes of the two inner petals. This association of pollen with the median lobe is purely fortuitous, but it may, unconsciously, have suggested to Eichler the comparison of this lobe with an anther—a comparison which enables him to harmonise the petal structure with his view of the stamen phalange of the *Fumarioideae*, on the assumption that the lateral lobes of the petals are equivalent to monothecal anthers.

I have examined the corolla in serial sections in order to see if there is anything in the structure to support Eichler's view. The inner petals are shown in their basal region for *H. leptocarpum* in Fig. 6, B₁₈, p. 155; *H. procumbens*, Fig. 10, B₅, p. 160; and *H. pendulum*, Fig. 11, A, p. 162. The extreme base of the median lobe is drawn for *H. leptocarpum* in Fig. 6, B₁₇ and B_{17a}, p. 155, while the median lobe, above the level of the lateral lobes, is shown in B₁₉. It will be seen from these diagrams that there is nothing in the structure of the petals which in any way favours Eichler's idea of the equivalence of the median lobe to a dithecal anther, and the lateral lobes to monothecal anthers; so I think that this theory may be dismissed, and that the petals may be taken to be in fact as they are in appearance—normal petals. Their peculiar form is probably to be related to the compression in the antero-posterior plane of the flower, to which we shall refer later (p. 171). On this view the young (inner) petals are as it were moulded by being clamped during their development between the back and front sepals and the essential organs.

(ii) *The staminal glands*

A feature of the flower of *Hypecoum*, which is particularly striking in serial sections, is the glandular development in the base of the filaments, described by Eichler as "eine drüsig-callöse Anschwellung⁽¹⁾." The general arrangement of the glands will be understood for *H. leptocarpum* in Fig. 6, B₁₈, p. 155. The gland tissue is localised at the filament margins, except at the extreme base, where the two patches of gland tissue are connected dorsally. As the section drawn in B₁₈ is slightly oblique, the left-hand stamen is cut at a lower level than the others, in the region in which the gland tissue forms a connected zone. The same basal region is seen in greater detail in Fig. 5, B₁₅, p. 153. It will be recognised from this drawing that the glandular tissue is formed of files of cells whose radial arrangement indicates meristematic activity. The cells are densely filled with cytoplasm and have conspicuous nuclei. The glands of *H. pendulum* are shown in Fig. 11, p. 162, and those of *H. procumbens* in Fig. 10, B₅ and B_{5a}, p. 160. It will be noticed, especially in Fig. 10, B_{5a}, that with the glands there are associated certain strands of elements which have no dense cell contents, and whose walls are often angular and sharply defined. These strands, which presumably serve for conduction, are not always easily distinguishable from the surrounding tissue, and their origin is generally difficult to trace. But in Fig. 4, B₁₄ and B_{14a}, p. 151, which are drawn from one of the outer stamens, it can be seen that in *H. leptocarpum* they are derived from the protophloem of the stamen bundle, after this bundle has been formed by the fusion of two strands. In Fig. 11, B₁-B₄, p. 162, which represents a stamen of *H. pendulum*, the gland strands can again be traced to the phloem of the stamen bundle, but here the strands for the two glands arise from the phloem of the two constituent bundles, before they fuse into the single stamen bundle. In this species I have also seen a second mode of origin for the gland strands; they may arise from the tangle of protophloem hyphae which exists in the receptacle below the detachment of the sepals. This type of connection of the gland strands with the main vascular system is also found in *H. procumbens*. The gland strands in this species are not at all easy to follow, but the one marked * in Fig. 10, B₅, p. 160, can distinctly be traced to a protophloem branch arising from one of the gynaeceum bundles (Fig. 9, B_{3b}, p. 158). The difference between the two modes of origin of the gland strands—from the phloem of the stamen bundles, or

directly from the phloem nexus in the receptacle which arises in connection with the gynaeceum strands—may be more apparent than real; for in *H. leptocarpum*, in which it is the phloem of the stamen bundle which supplies the gland strands, the stamen bundles themselves receive their phloem from the x bundles which are also responsible for the phloem nexus.

As a rule in the gland strands, elements which are empty, or have colourless contents, are surrounded by others which are densely filled with cytoplasm, so that the strand is rosette-like in section. The cell walls of these protophloem elements are thin, but more sharply stainable than those of the surrounding glandular tissue (Fig. 11, B₃). When the strand has actually entered the gland, it becomes difficult to distinguish those of its elements which have dense cell contents from the gland cells.

So far as the nature of the gland strands is concerned, the *Hypecoideae* fall into line with the groups already investigated. In the *Fumarioideae* (4), p. 342 I have shown that, in *Corydalis*, *Fumaria* and *Dicentra*, the nectariferous region is served by phloem strands given off from the stamen bundles. In the *Cruciferae* (2), p. 30, again, the glands are supplied by protophloem strands, but these strands are even more variable in their origin than are those of *Hypecoum*.

(iii) *The phloem nexus*

In *Hypecoum leptocarpum* the phloem strands of the four bundles (marked x in Fig. 2, B₆, etc.), whose xylems give the xylems for the gynaeceum bundles, show complexities of behaviour which were scarcely touched upon in the descriptive section of this paper. These strands branch irregularly, and their subdivisions, anastomoses, and changes of direction produce a three-dimensional tangle of phloem fibrils which defies reduction to any definite system. Fig. 8, C₁ and C₂, p. 157, gives some idea of the complication of the phloem associated with the gynaeceum, even at the level at which the xylem strands have settled down to their final arrangement. The erratic behaviour of the phloem system is illustrated by the fact that one of its strands passes horizontally across the centre of the receptacle between the main vascular bundles (Fig. 8, C₂ and C_{2a}). Similar phloem complexities may be observed in *H. procumbens*. In Fig. 9, B₂ and B₃, p. 158, phloem wefts are seen between the bundles; they arise in the main from the strands which, at a higher level, will be associated with the gynaeceum. One of the gynaeceum bundles is drawn on a larger scale in B_{3a} to show the horizontal and vertical

branching of the protophloem; the vertical branches are often rosette-like in section.

In the account of the staminal glands (p. 164) I have emphasised the intimate relation which exists between the phloem nexus, on the one hand, and the gland strands and the phloem of the stamen bundles on the other. But apart from its functional importance, this nexus has a significance in connection with the morphological use of anatomical data; I hope in a later paper to consider it from this point of view.

(iv) *The analysis of the androecium*¹

Hildebrand in 1870(9) recorded that the two stamens of *Hypocoum procumbens* opposite the inner petals were two-bundled, and he considered that this confirmed Eichler's view of the morphology of the androecium(6). According to Eichler, in the Fumitories the lateral monothecal members of the two stamen phalanges represent the stipules of the median dithecal members. In order to fit *Hypocoum* into this scheme, he supposes that the stipular members have left the lateral stamens to which they belong, and have fused in pairs in the antero-posterior plane, after the manner of interpetiolar stipules. In the *Blüthendiagramme*(7) he adheres to the view which he expressed in 1865, and he adds a floral diagram of *Hypocoum* incorporating Hildebrand's observation of the presence of two bundles in the inner stamens. But he diverges from Hildebrand's drawing in representing the anthers as bifid, and he states in the text that they are often deeply cleft. Čelakovský(5), though treating the monothecal members of the Fumarioideae phalange as independent stamens, yet agrees with Hildebrand and Eichler in regarding the inner stamens of *Hypocoum* as equivalent to the monothecal stamens of the Fumitories fused in pairs.

In reviewing the literature we find that the statements of fact, on which the views of Hildebrand, Eichler, and Čelakovský are based, have not passed unchallenged. Prantl and Kündig(11) write that they can nowhere find the doubled vascular bundle in the median stamen of *Hypocoum*, to which so much importance is attributed by Hildebrand, and they assume that it must have been an exceptional occurrence.

It is clear, then, that before we can interpret the flower of *Hypocoum*, it is necessary to re-examine it in order to find out whether the

¹ For further details of the views which have been held on the subject see Fedde(8).

following points in previous descriptions can be accepted: (a) the cleft form of the inner anthers, described by Eichler(7); (b) the two-bundled condition of the inner stamens, described and figured by Hildebrand(9). I have tried to settle these two points by means of serial sections of the flowers. (a) I have followed the anthers to their apices, and I have found that, in two-bundled stamens of *H. leptocarpum* and *H. procumbens*, there is no bifurcation separating the thecae in the undehiscent anther. This is shown by the sections near the tips of anthers drawn for *H. leptocarpum* in Fig. 6, B₁₉a, p. 155, and for *H. procumbens* in Fig. 10, D₂, p. 160. I suppose that Eichler examined the stamen as a solid object, and he may perhaps have been deceived by the fact that the anthers ripen and dehisce at a very early stage¹, so that, when the flower opens, their natural form is already obscured. (b) As regards the second point—the observations recorded in the present paper account, I think, for the discrepancies between previous descriptions on the question of the two- or one-bundled condition of the inner stamens. For though the inner stamens of *Hypecoum leptocarpum* are usually two-bundled (Fig. 6, B₁₆ and B₁₉, p. 155) they may be one-bundled (Fig. 8, D, p. 157); though those of *H. procumbens* are generally one-bundled (Fig. 10, B₅, p. 160) they may be two-bundled (Fig. 10, D); while in the few flowers I examined of *H. pendulum*, I found no exception to the one-bundled state (Fig. 11, A, p. 162).

The position, then, as regards the actual facts of the stamen structure is that the inner stamens of *Hypecoum* are sometimes two-bundled and sometimes one-bundled, but that they are not deeply bicleft as described by Eichler. The next question is how these facts are to be interpreted.

It is certainly true that the replacement of two adjacent stamens by one stamen with a two-bundled filament is a possible occurrence. To show that this does occasionally happen, I have included here some sketches of the inner stamens from an abnormal flower of a young inflorescence of the watercress, *Nasturtium officinale* R.Br., in which this replacement has actually occurred. The stamen pair to the south is supplied by two bundles (Fig. 12, B, p. 168), but these two bundles enter one filament (C). The anther (E) shows its duplex origin clearly, the adjacent pollen-sacs of the two anthers forming a single cavity. In the northerly pair, the union of the two stamens is more complete. A single bundle enters the filament, and in such an

¹ According to Hildebrand(9) dehiscence occurs "wenn die Knospe noch sehr klein und noch ganz grün ist."

anther section as C, the double character is only indicated by the unusual width of the bundle. In D, however, the bundle has divided into two, and there is a fifth pollen-sac. This example shows that the theory that the two-bundled stamens of *Hypecoum* each represent two stamens cannot be dismissed *a priori*. But this does not mean that it is necessarily correct. The question of the degree of probability that attaches to it can only be answered by carrying the study of the anatomy further than was possible in the pre-microtome days when this theory was suggested. The crucial point is to find out by means

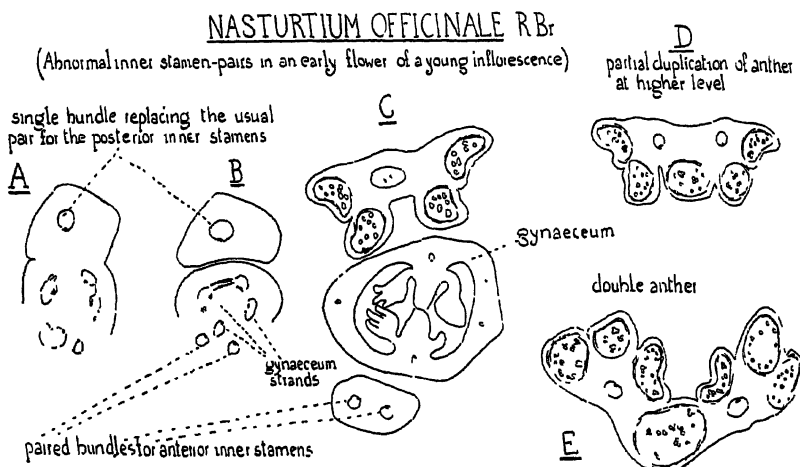


Fig. 12. *Nasturtium officinale* R.Br. Transverse sections ($\times 56$) through an early flower, Coe Fen, Cambridge, May 27th, 1930. A and B, partial transverse sections of a receptacle showing the bundles for the anterior and posterior stamens. C, gynaecium, and the fused stamens which replace the anterior and posterior pairs. D and E, the anthers for the northerly and southerly fused pairs.

of serial sections what is the actual origin and course of the stamen bundles. Much of the present paper has been devoted to following out their history. I will only now refer in the briefest way to the results of this examination; the details will be found on pp. 146-163. In *Hypecoum leptocarpum* there is a level in the receptacle of the flower in which the stamen system includes eight xylem groups (Fig. 7, 1, p. 156). For the east and west stamens these xylem groups fuse in pairs; the process of fusion can be followed in Fig. 5, (1)-(4), p. 153. Those which enter the north and south stamens, on the other hand, remain free, so that the result is two two-bundled stamens, and

two which are one-bundled (Fig. 7, 3, p. 156, and Fig. 6, B₁₈ and B₁₉, p. 155). In *H. procumbens* we again meet with an eight-bundled stage below the detachment of the stamens. Six of the eight xylem strands are visible in Fig. 9, B₃ (*Stc''*, *Stb''*; *StB'*, *StB''*; *StD'*, *StD''*), while the two remaining strands to the south come into view in Fig. 10, B₄. In *H. pendulum* the structure is similar. But in *H. procumbens* and *H. pendulum*—in contrast to *H. leptocarpum*—the eight stamen bundles all fuse in pairs, so that the four stamens have each one bundle only.

It will be seen from this account, that whatever meaning is attached to the two-bundled character of the inner stamens, *involves also the outer stamens*, whose vascular system equally takes its origin from two bundles, though this fact is obscured by secondary fusion which occurs before the bundles enter the filament. On Hildebrand's view, if it is carried to a logical conclusion, the (apparent) four stamens of *Hypecoum* must thus be interpreted as being actually eight, fused two by two. And if the eight androecium bundles are thus each regarded as the representative of a stamen, the same view should be applicable to other corresponding cases. For instance in *Dicentra spectabilis* Lem. (Fumarioideae) the median bundle for the lateral petal and that for the superposed stamen (the dithecal member of the phalange) arise from one bundle which branches into three, the median member entering the petal, while the wing members fuse in front of the median member and enter the stamen. This process can be followed in (4) Fig. 10, B₃ and B_{3a}, p. 335, and Fig. 11, p. 337. If the *Hypecoum* stamen is interpreted as double, the central member of the stamen phalange in *Dicentra* must hence be regarded as double also. And the same reasoning leads, moreover, to the conclusion that the *inner petal* of *Hypecoum* is a duplex structure, for the median bundle of this petal—exactly like that of the stamen—arises by fusion of two bundles, as shown diagrammatically for *H. procumbens* in Fig. 10, C, p. 160. No doubt—given the necessary ingenuity—the flower of *Dicentra* could be explained on the theory that the central member of each stamen phalange is really a pair, while that of *Hypecoum* could be interpreted on the assumption that not only each stamen but each inner petal is two-membered. But it is a mistake to resort to complex schemes of a purely hypothetical nature unless no simpler solution offers itself. If we ask what simpler interpretation can be found to account for the double vascular supply of the stamens of *Hypecoum*, the answer is, I think, that *this double character is merely a direct result of a particular mode of bundle branching associated with*

the superposition of a stamen to a petal. In this case, one bundle is responsible for the main vascular system of an outer petal and its superposed stamen. This bundle, as we have said, divides into three, the median member serving the petal, and the two laterals fusing in front of the median member to form the stamen bundle¹. In the inner stamens the same principle has to be carried further, because one bundle supplies not only a petal and its superposed stamen, but also a sepal on the same orthostichy. A triad division first occurs, corresponding to that which produces the bundles for the outer petal and outer stamen, but this is followed by a second division into three on the part of the wing bundles; of the bundles thus produced, one pair fuse in front of the sepal bundle to make a petal midrib, and a second pair either fuse in front of the petal for the stamen, or remain free. These branchings can be followed in the A group to the north in Fig. 9, B₃, p. 158, and they are shown diagrammatically in Fig. 10, C, p. 160. This mode of division offers a simple and symmetrical solution of the problem of producing superposed collateral bundles from a single collateral bundle, which cannot—without profoundly reorganising its structure—give rise, by means of tangential division, to collateral daughter bundles lying on the same radius of the axis as itself. And it seems to me not unlikely that future research may reveal the recurrence of this mode of bundle branching in other families in which stamens are superposed to petals.

If we accept this view of the meaning of the paired stamen bundles of *Hypecoum*, the next question is whether any reason can be suggested for the fact that the bundle pairs sometimes fail to fuse. I believe that the answer is provided by considerations of space and position. So far as my observations go, fusion always occurs in the outer (lateral) stamens, though not always in the inner stamens. I think that this difference is probably due to two factors—one of which is concerned with a slight distinction between the vascular systems of the two whorls, and the other with the general symmetry

¹ Branching of this type recalls the characteristic bundle relation which one can often trace between a young axillant leaf and its axillary bud; one of the shoot bundles divides into three parts—the median member serving the leaf, while the wing members approach one another on the inner side of the median member, and supply the bud. I have illustrated an example of this common method in Fig. 1, A₃–A₅, p. 147, and it has been previously sketched for certain Fumariaceae ((4), Fig. 2, D₁, p. 321, *Corydalis nobilis*; Fig. 3, A₁–A₅, p. 323, *C. bulbosa*). This close analogy between the mode of bundle connection of a leaf and its axillary bud, and that of two leaf members on the same orthostichy, points to the unsatisfactory character of “stem” and “leaf” as categories (see(1)).

of the flower. In the first place it must be remembered that the bundle pair of each *outer* stamen is formed by two branches of a triad division of a single bundle, while the bundle pair of each *inner* stamen consists of two branches resulting from a later division which succeeds the corresponding triad division. The inner stamen bundle pairs are therefore not quite so closely connected as the outer stamen bundle pairs, since they are granddaughter instead of daughter bundles of the main trunk; approximation and fusion may thus be a degree less easy. To understand the second factor, we must turn from the details of the vascular system to a consideration of the flower as a whole. As there are only two sepals (at the back and front of the flower), the later floral whorls tend to be somewhat compressed between them, so that the width of the flower is greater than its depth in the antero-posterior plane (cf. Fig. 6, B₁₉, p. 155). The stamens, in common with the other parts of the flower, are affected by this flattening, which expresses itself in a marked difference between the width of the inner and outer filaments—those of the inner stamens being the wider. It is not unlikely that the greater filament width of the inner stamens promotes the freedom of the bundles.

If this idea be sound it may perhaps be used in attacking a further problem—why it is that in *H. leptocarpum* the inner stamens remain two-bundled, while in *H. procumbens* and *H. pendulum* there is fusion into a single strand. Simple inspection is enough to determine the broad fact, to which I have already referred, that in all three species the inner stamens are wider than the outer, but when it comes to comparing the species among themselves, more exact methods are needed. In the attempt to get some definite expression for the relations of the bundle widths, I have measured, in my sections, the bases of the filaments of those flowers which were cut in a plane near enough to the horizontal to give some degree of exactness to the figures. I found that in nine flowers of *H. procumbens* with one-bundled inner stamens, if the average width of the base of the *outer* filament were taken as the unit, the average width of the *inner* filament was between 1.2 and 1.3; while in four flowers of *H. pendulum*, also with one-bundled inner stamens, their average width was between 1.3 and 1.4. In *H. leptocarpum*, on the other hand, the measurements of four flowers gave an average of between 1.6 and 1.7 for the inner stamens. It is obvious that caution must be used in drawing conclusions from so small a number of measurements, but I think that we are justified in saying that *H. leptocarpum*, the species in which the inner stamens are two-bundled, has a greater relative width of

inner filament than *H. procumbens* and *H. pendulum*—species in which the inner stamens are one-bundled. This comparison of the inner stamens alone, from species to species, thus confirms the result already reached by comparison of the outer and inner stamens in the three species taken together—that the greater the width of the filament, the more likely are the two bundles to retain their individuality.

4. SUMMARY

In the present paper the flower structure of the Hypecoideae is considered. The other sub-family of the Fumariaceae—the Fumarioideae—has already been described(4). From a study of *Hypecoum leptocarpum* Hook. f. et Thoms. (pp. 146–57 and Figs. 1–8), *H. procumbens* L. (pp. 158–61 and Figs. 9 and 10), *H. pendulum* L. (p. 161 and Fig. 11) and *Chiazospermum erectum* Bernh. (pp. 161–63), the following conclusions are reached:

1. The structure of the inner petals of *Hypecoum* lends no support to Eichler's view that these petals are each equivalent to a stamen phalange of the Fumarioideae (p. 163).

2. In *Hypecoum* each filament has a development of glandular tissue near the base, mainly confined to the margins. These glands are supplied by delicate phloem strands, recalling those which enter the glands of the Fumariaceae(4) and Cruciferae(2). The origin of these strands is not easy to trace, but I have found that they may be derived from the phloem of the stamen bundle or from a phloem nexus which is formed in the receptacle (pp. 164–165).

3. Hildebrand(9) described the inner stamens of *Hypecoum* as two-bundled, but other writers have been unable to confirm his observation. I find, however, that this discrepancy is accounted for by the fact that the one- or two-bundled character varies from species to species, and even from flower to flower in the same inflorescence. Hildebrand(9), Eichler(6) and (7), and Čelakovský(5) have taken the view that the two-bundled inner stamens of *Hypecoum* each represent two members—ancestrally free, but now in a state of fusion—while the outer one-bundled stamens are truly single; their general interpretation of the flower is based upon this theory. But I find that the two-bundled character of the stamen supply in *Hypecoum*—which is common to all four stamens, though masked in the outer, and sometimes in the inner whorl by subsequent fusion—is due merely to a special type of bundle branching, associated with the superposition of stamen to petal. There is thus no need to invoke the survival of

anatomical relics of ancestral structures in order to explain it. Whether the bundle pairs retain their independence or fuse, appears to depend—at least in part—upon the relative width of the filament base (pp. 166–172).

CAMBRIDGE,
November 26th, 1931.

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Since this article was in print, I have received flowers of *Pteridophyllum racemosum* Sieb. et Zucc. by the kindness of Mr W. E. T. Ingwersen. So far as can be judged from a rapid examination, the stamens, which seem to be all one-bundled, differ from those of the other *Hypocoidae* in alternating with the midribs of the petals instead of being placed opposite to them. These two characters, considered in relation to one another, lend indirect support to the view expressed above, that the two-bundled character of the filaments of *Hypocoum* is an outcome of the superposition of stamens to petals.

ON SOME RECENT CONTRIBUTIONS AND CRITICISMS DEALING WITH MORPHOLOGY IN ANGIOSPERMS

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(With 27 figures in the text)

IN a communication which treats of the vegetative members of the plant⁽³⁾ and in the first three papers of a projected series on the subject of floral morphology (1, 5, 6), A. Arber sets forth the grounds upon which, on the one hand, she now finds herself constrained to abandon her adhesion to the Leaf-skin theory of the stem (which she had earlier accepted); and, on the other hand, to adhere to the traditional bicarpellary view of the gynoecium of the Cruciferae and the Fumarioideae, at least to the extent of retaining it "as an instrument of description¹." Some reply to this writer's arguments and pronouncements regarding the conceptions involved both in the theory of the Leaf-skin and the theory of Carpel Polymorphism seems called for on several grounds. In the first place, because some of the evidence and arguments put forward do not appear to justify the conclusions based upon them. Secondly, because the theory of Carpel Polymorphism and its implications are so misapprehended as in some instances to suffer serious misrepresentation, expressed or implied.

To deal with every structural feature discussed in these several accounts would extend this commentary to unmanageable length. Portions of the two papers on the Cruciferae confirm points already established; these I may pass by. For the rest, without following this writer into the realm of metaphysics, I hope to show that her argument for regarding the conception of the leaf-skin as superseded by that of the antithetic relation of root and shoot is illogical; and to

¹ The two earlier papers in this series treat of the Cruciferae, the third of the Fumarioideae. The circumstance that the earlier portion of the present account dealing with the first two papers of the series had already been completed before the third appeared has made it, unfortunately, inevitable that the two families should be considered separately.

make clear that no evidence offered by her is incompatible with, or controverts, the theory of Carpel Polymorphism.

I. THE VEGETATIVE MEMBERS AND THE THEORY OF THE LEAF-SKIN

We recognise in the spermatophyte shoot an axis on which may be borne a number of lateral members, differing as a rule in appearance from the axis bearing them, arranged in varying manner in different types, but in ordered sequence according to definite schemes. Formerly the free portion of each of these unit members had been generally regarded as constituting the whole of that member. The fundamental conception underlying the theory of the Leaf-skin is that the exerted structure is only the upper portion of each such unit, the lower portion, extending downwards below the level of exertion, being fused on its inner face with the axis core, and at its edges with its neighbours (15). We might indeed compare the shoot to a periclinal (stem-leaf) chimaera. Now, it may (or may not) be, that in the future we shall obtain new evidence throwing light upon the phylogenetic relations of stem and leaf, and in consequence we may (or may not) adopt a new terminology. But such verbal changes will not alter those observable relations expressed in the theory of the Leaf-skin, which views the shoot as a core clothed by a mosaic.

A. Arber urges that the Leaf-skin theory does not define the boundary between "skin" and "core." Why should we expect to be able to discern any such internal demarcation? Do we find any between the fused bases of corolla and androecium in cases where stamens are epipetalous? Do we find such definite boundaries anywhere in any complex organism between different regions of the same continuous tissue? Even in the case of the units which this writer would alone have us recognise, viz. root and shoot, there has been shown to be an interpenetration of the vascular elements of the two organs. The cortex of the two members shows no boundaries. Only at the surface, if anywhere, can a dividing line be traced. That is to say, the boundaries of root and shoot are no better defined than those of stem and leaf. Is it then logical to retain the major categories of root and shoot and not to recognise the minor ones of stem and leaf? In the end this writer traces back her argument to no basis of concrete fact but to the conception of an abstract entity—the cauloid—which has no existence outside the imagination. And there this writer leaves the question. But the actual living shoot still confronts

us, and in the elucidation of one of its characteristic features the Leaf-skin theory has already proved fruitful. This theory has enabled us for the first time to grasp the significance of the various observed schemes of phyllotaxis, which, so long as the exserted structure was regarded as the whole of the leaf unit, remained unexplained. We are now able to see that, as in each species there is an inherited tendency to develop leaves having a certain exsertion width in relation to the circumference of the axis, it is this relation which underlies the particular arrangement of the exserted portion of the leaves, and the particular Leaf-skin pattern (where traceable) produced by the non-exserted portions. It may be recalled that A. Arber herself would seem to have arrived at an understanding of the relations of the *squamae intravaginales* occurring in the Helobiae and some of the Araceae by way of the Leaf-skin (1), p. 96). It does not appear how these relations would have become apparent if no other categories were recognised than root and shoot. The only logical position appears to be *either* to regard the leaf-bearing plant as an indivisible whole *or* to recognise categories of higher and lower order.

A further argument developed by A. Arber in the course of her discussion on the relations of stem and leaf sets out to show that, contrary to the generally accepted view, the axis may terminate in a leaf (3), p. 302). This point is of more than theoretical interest owing to its bearing on the question of the composition of those terminal ovaries which have in the past been regarded as monocarpellary. According to this writer, the appearances seen in transverse sections of the flowering region in certain bamboos prove that here the axis ends in a flowering glume. The question that arises is whether these appearances invalidate the generalisation that the stem axis never terminates in a leaf. It seems to me quite clear that they do not. The typical grass leaf, as we know, has a circular exsertion; it has the form at first of a hollow cone. This, from A. Arber's figure (2), p. 185, Fig. 5, A₄), is seen to be the case with the flowering glume in question—as we should expect. Now a structure which is hollow at its base from the moment of origin clearly cannot be described as terminal on the axis bearing it. There may be no discernible development of the axis above the exsertion level of the glume, but that is beside the point. A ring base by its nature cannot be derived from the apical cells in the centre line of the axis. But it is from the centre line cells that development proceeds in a structure which is truly described as terminal. This mode of development takes place in the continued growth of the vegetative axis and in cases of proliferation in the

flower. It does not take place, so far as we know, in the case of any foliar member of Monocotyledon or Dicotyledon. Nor does it seem probable that any such case will be found, for in the Monocotyledon the leaf exsertion is typically circular, in the Dicotyledon it is typically lateral. If in a seedling plant of date or onion it should happen for some reason that the plumule remained undeveloped, this condition would not justify us in regarding the cotyledon as a terminal structure in the sense implied in the generalisation "that a leaf does not terminate an axis." But it is just such postulated relations that are exhibited in miniature in the bamboo spikelet. What, therefore, it appears to me is to be deduced from A. Arber's observations is not that they disprove one of the generalisations of what she describes as "formal" morphology, which, be it remembered, have their foundation in the observation of facts, but that they furnish further evidence that, despite outward appearance to the contrary, this generalisation still remains true. (For further consideration of this subject see later, p. 205.)

We may now turn to the consideration of various points discussed by this writer in her account of the morphology of the flower.

2. FLORAL MORPHOLOGY AND THE THEORY OF CARPEL POLYMORPHISM

Cruciferae

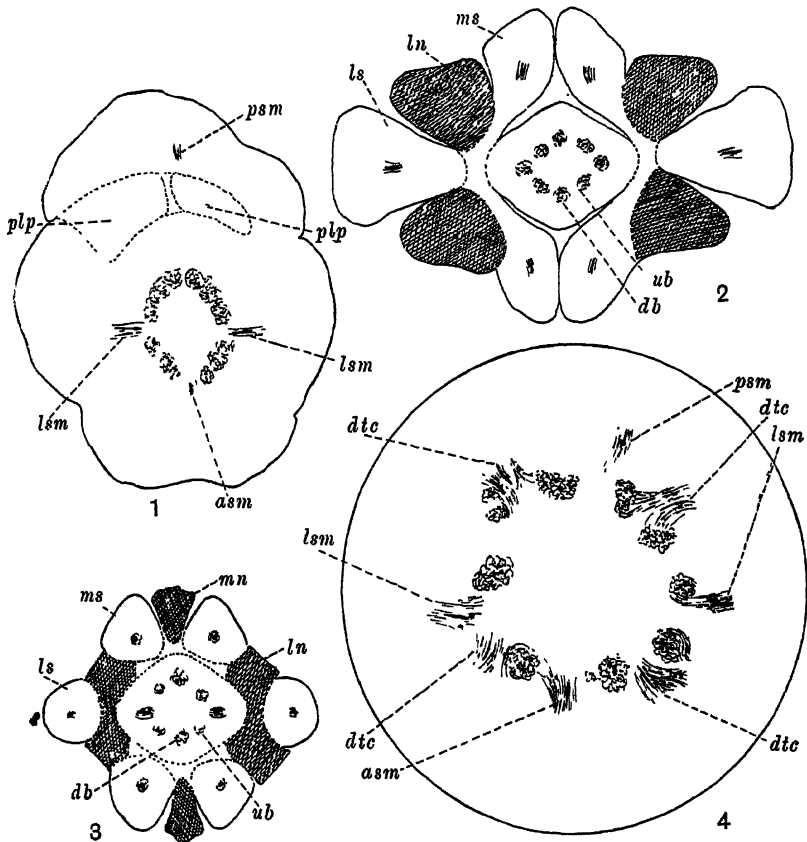
(a) *The calyx.* In treating of the calyx, A. Arber adduces certain evidence regarding the difference in level at which the midrib bundles of the median and lateral sepals turn outwards from the central cylinder in the types investigated by her. She points out also that in cases where the midrib bundles for the lateral sepals emerge before those for the median members, and where also the lateral sepals are the first to be exserted, their margins may nevertheless be overlapped by those of the median pair, thus leading to the false appearance of the lateral members being in such cases the inner pair. This writer further directs attention to the consideration that where, as in the Cruciferae, the bracteoles are completely suppressed¹ it would be in accord with expectation that the sepals standing in the lateral plane should be the outer members. The above statements

¹ The bract is also in most cases suppressed so far as the exserted region is concerned, but the non-exserted portion (the Leaf-skin component) is developed like that of the leaf in the vegetative region.

give occasion for the following comments which have a bearing on certain points which will be discussed later.

In the first place it may be observed that the fact that in some cruciferous types, as e.g. *Cheiranthus*, the median sepals overlap the lateral members and in others, as noted by A. Arber, are overlapped by them, is in itself an indication that the four sepals constitute a single whorl, not two. For no instance is known, so far as I am aware, where the outer of two perianth whorls in one type becomes the inner in another type belonging to the same family and having the same bract arrangement and the same orientation. On the other hand it is by no means rare to find, in similar circumstances, that the mode of aestivation in the *same* whorl varies from type to type.

Secondly, in considering the significance of the early departure from the central cylinder of the midrib bundles for the lateral sepals in those cruciferous types investigated by A. Arber and in many others, certain other features must be taken into account, in particular, the number, size and distribution of the nectaries. These glands are generally developed beside or around the lateral stamens. If others are present in addition in the median quadrants of the flower they are usually less prominent. It can scarcely be doubted that the pouched shape of the lateral sepals, the depressed level at which their midrib bundles leave the central cylinder, and the earlier exertion of these members, where these features occur, are all adaptations to provide the necessary space for the nectaries in the lateral quadrants. In illustration of this point we may compare the two types *Sisymbrium Alliaria* Scop. and *Berteroa incana* DC. In *Sisymbrium* (Fig. 3) we have a type in which the nectaries are of such size, and so situated, that no considerable modification is needed for their accommodation. Four glands are present. All four lie within the contour line of the androecium. Two are situated in the median plane between the bases of the adjacent inner stamen filaments back and front; two, in the form of crescents, partly encircle the bases of the lateral stamens on their inner side. This distribution necessitates the provision of much the same accommodation in the lateral and in the median planes. Hence it is not surprising, in such circumstances, to find here a calyx of four approximately equal sepals arising at the same level. In the accompanying drawing of a transverse section through a bud of this species taken from a late-flowering individual (Fig. 4) it will be seen that the bundles for the midribs of the four sepals take their rise from the central cylinder simultaneously. The uniformity in level is of the same order as that of the four trunk cords

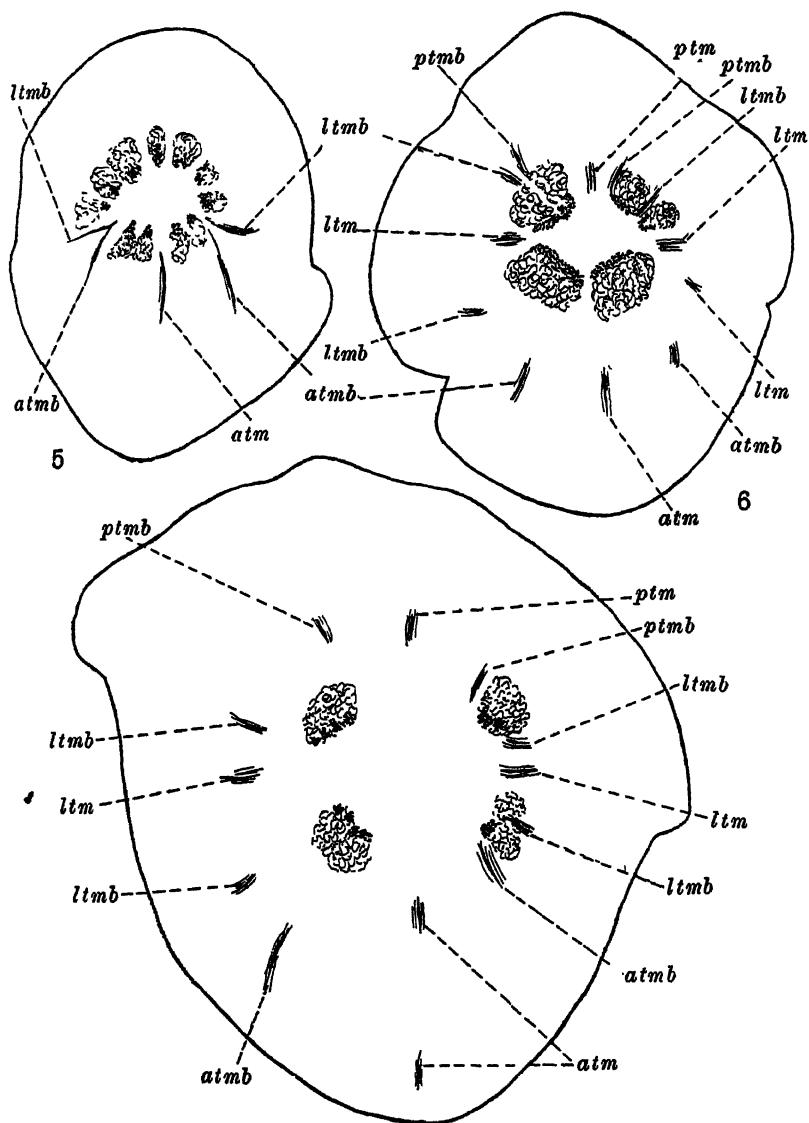


Figs. 1-4. All from transverse sections. 1. *Ericaceae*. *Erica grandiflora* L. Flower base. At the back the posterior sepal about to become exserted; beneath it portions of the two postero-lateral petals already defined but not yet disjointed. In the centre the residual vascular ring from which the bundles for the two lateral sepals have already turned out for some distance; the bundle for the anterior sepal is just beginning to emerge. 2-4. *Cruciferae*. 2. *Berteroa incana* DC. The flower after exsertion of the calyx and corolla. To right and left a protruding lateral stamen and a pair of nectaries. In the centre four orthogonal differentiated vascular cords destined for the carpels and four intervening undifferentiated strands. 3. 4. *Sisymbrium Alliaria* Scop. 3. The flower as in 2 showing median as well as lateral nectaries. 4. Base of a flower bud at the level at which the sepal midrib bundles and the trunk cords for the sepal marginal veins and petal midribs leave the central cylinder.

asm, anterior sepal midrib; db, differentiated bundle; dte, diagonal trunk cord; ln, lateral nectary; ls, lateral stamen; lsm, lateral sepal midrib; mn, median nectary; ms, one of the four diagonal stamens which converge to form two median pairs; plp, postero-lateral petal; psm, posterior sepal midrib; ub, undifferentiated bundle.

furnishing the petal midribs, which here follow almost immediately on the alternate radii¹. Clearly the four sepals form a single whorl just as do the petals, and this relation is confirmed, as we shall see presently, by other evidence. On the other hand, in *Berteroa incana* DC. (Fig. 2), where there are no nectaries in the median quadrants but a large projecting pair in each lateral quadrant which cause the lateral stamen filaments to protrude considerably, the lateral sepals are correspondingly depressed. It is evident that in *Berteroa* and similar types deformation of the single calyx whorl takes place in accord with an asymmetrical nectary development which, rather than the absence of bracteoles—a feature common to *all* types—must be regarded as the determining factor where the lateral sepals are depressed. We meet with a more extreme case of such deformation through unilateral nectary development in *Corydalis* (see Figs. 24–26), where the midrib bundle for the one-spurred lateral petal leaves the central cylinder at a very much lower level than the bundle for the opposite unspurred petal, though the two petals are members of the same whorl. Cases in which, in contrast with the Cruciferae and Fumarioideae, deformation of the whorl is properly attributed to the bract arrangement are to be met with, however, among tetramerous species of *Erica* (*E. grandiflora* L., *E. stricta* Donn). In these forms, in which both bract and bracteoles are well developed, the bundle for the posterior sepal midrib leaves the central cylinder very much earlier than the corresponding bundles for the three other sepals which are superposed on the three bract members (see Fig. 1). Again in certain tetramerous types belonging to the section Rivineae of the Phytolaccaceae, as e.g. *Rivina humilis* L. (Figs. 5, 6) and *Ledenbergia roseo-aenca* Lem. (Fig. 7), the midrib bundles for the four perianth members of the single perianth whorl arise at different levels. Here, however, the order of development is the reverse of that in *Erica*. Although bract and bracteoles are present in both *Rivina* and *Leden-*

¹ A. Arber gives a figure of this same species ((4) p. 13, Fig. 1, 2) in which the bundles for the lateral pair of sepals are shown already at some distance from the central cylinder, while those for the median members are only just emerging. In my figure of an earlier stage (Fig. 4) it will be seen that it would be impossible to tell which bundles were destined for the median and which for the lateral pair, the level of origin of all four being the same at the bud stage represented. It may be that individuals show variation in this respect; but it seems more probable that a difference in level develops rapidly as growth proceeds. But if such be the case it would merely afford additional proof that the deformation is secondary and that the whorl is single. This case furnishes an answer to the doubt expressed by the above writer ((4), p. 28) as to whether "there are any Crucifers in which the bundles for the two pairs of sepals emerge *precisely* at the same level."



Figs. 5-7. Phytolaccaceae. All from transverse sections. 5, 6. *Rivina humilis* L. 5. Flower base at the level at which the midrib bundle for the anterior tepal is leaving the central cylinder; on the diagonal radii to right and left a pair of issuing strands. Each pair furnishes the marginal vein of the tepal to right and left respectively. 6. The same at a later stage. The midribs and marginal veins of all four tepals are now differentiated. 7. *Ledenbergia roseo-aenea* Lem. Flower base showing the same features as in 6.
 atm, anterior tepal midrib; atmb, anterior tepal marginal bundle; ltm, lateral tepal midrib; ltmb, lateral tepal marginal bundle; ptm, posterior tepal midrib; ptmb, posterior tepal marginal bundle.

bergia, though small and without vascular tissue, it is here the bundle for the *front* perianth member which turns out first from the central cylinder. This is followed after an appreciable interval by those for the two lateral members, the bundle for the back member lagging slightly behind these last. Yet *both* the median members overlap the lateral pair. These differences do not affect the fact that the four members exhibit the relations of a single whorl, and we therefore express these relations in *Erica*, *Crucifer* and *Rivineae* by K_4 and T (tepal) 4 respectively, not by $K_1 + 3$, $K_2 + 2$, and $T_1 + 2 + 1$. Though this distinction may seem of little moment in itself, it becomes a matter of some consequence when we come to weigh the arguments in favour of a tetramerous ground plan for all whorls in the *Crucifer* flower, and it is for this reason that I have dealt with this question at some length.

That no generalisation as to the expectation in regard to the position of the members of a tetramerous perianth, such as that cited above by A. Arber, can be founded on bract arrangement alone is illustrated by another member of the *Rivineae*. In *Petiveria alliacea* L., as in *Rivina* and *Ledenbergia*, bract and bracteoles are present, yet in *Petiveria* the four perianth members stand in the diagonal planes, whereas in both *Rivina* and *Ledenbergia* they lie in the orthogonal planes. This difference in ground plan in such closely related forms has always been a puzzle. We find the answer in a corresponding difference in the vascular anatomy. In *Petiveria*, in which the bract and bracteoles are close to the flower, *all three receive a midrib bundle*. These bundles lie in the median and lateral planes respectively. The result here is that the midrib bundles for the first floral (perianth) whorl take up their position in the alternate radii, i.e. in the diagonal planes. In the two other above-mentioned genera bract and bracteoles are alike *non-vascular*. The first bundles to leave the central cylinder, as before, issue in the orthogonal planes, but here they enter, not bracts, but perianth members which thus stand in these planes.

Another feature of the calyx which has an important bearing on the questions of whorl arrangement is the mode of origin of the marginal veins of the sepals. In A. Arber's account they are described as arising through the giving off of two branches from each (so-called) petal midrib bundle, one branch passing to the adjacent sepal on either side (4, p. 29). Regarded *merely as a description of what is to be seen*, this statement may pass. Regarded *as conveying a picture of what may be supposed to take place as development proceeds*,

it cannot be accepted. Here, as in A. Arber's discussion on the constitution of the gynoecium (see later, p. 199), it is made apparent that a terminology employed solely as an "instrument of description" without appreciation of the occurrences underlying the appearances described, *may* be, and in the present case *is*, profoundly misleading¹; its effect is not to reveal the true situation but to obscure it. The following illustrations will serve to render this clear. Where, as in the Primulaceae, the stamens are epipetalous, we do not consider that a stamen midrib bundle gives off a *branch* which becomes the petal midrib bundle. So to describe the appearances would be to invert the natural order of evolution. Clearly what happens here is that a *trunk cord* leaves the central cylinder, i.e. a vascular cord in which are associated the elements for the two midribs which, as they differentiate, become dissociated. Again, where, as sometimes occurs among the Leguminosae, the midrib bundles of two or even three adjacent members of the 2-whorled perianth together with those for the superposed stamens emerge as a single trunk cord (see Figs. 8 and 9, also (20), p. 246, Fig. 66) we do not regard the petal midrib component as giving off a *branch* to furnish a neighbouring petal midrib, nor again do we describe the stamen component as giving off a *branch* to form the midrib of sepal or petal as the case may be. We consider that the elements for the different midrib bundles have emerged in conjunction as a single trunk cord². Yet again, in the case of those Piperaceae such as *Peperomia* in which each cord that leaves the central cylinder of the flowering axis furnishes the strands for the accompanying bract, the two stamens and the gynoecium we do not picture the carpel bundles as giving off *branches* to the bract and stamens which are older and outer structures, even though these bundles here continue upwards in the original direction while those for the bract and stamens turn off more or less at right angles. Examples of this nature might be multiplied *ad lib.* In all such cases the issuing vascular cord is clearly not a simple bundle. It is a complex not yet resolved into its components. I have elsewhere dealt at some length with the case of the marginal bundles of the sepals in the Caryophyllaceae and Primulaceae (23), and have drawn attention to the fact that these bundles arise in the same way in the Cruciferae

¹ Since complete avoidance of any implication is almost beyond attainment.

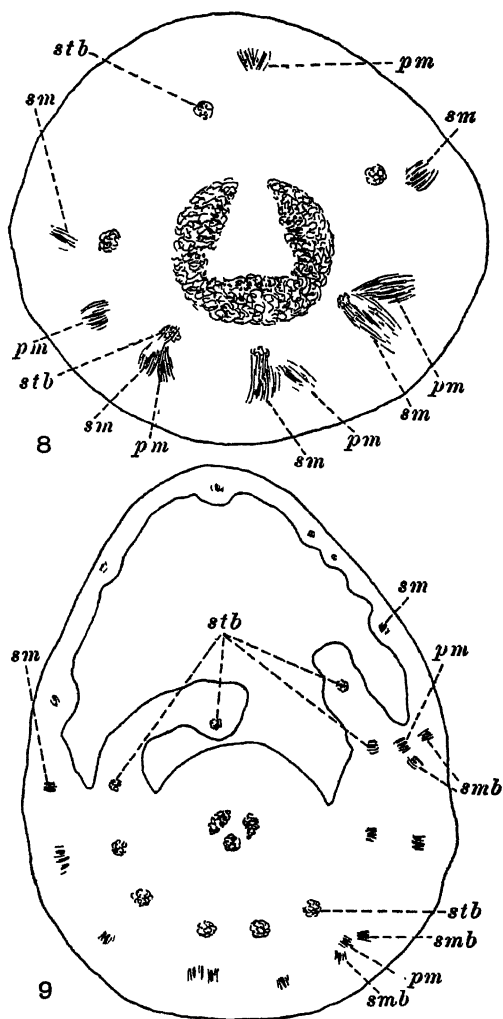
² Although it is sometimes convenient in treating of the trunk cords to refer to these cords as though their component elements were from the beginning organised into the strands into which the cords later become dissociated, it is most likely that, in fact, such organisation only becomes a reality at the moment of dissociation.

as in these two families. The same relations are also met with in the Leguminosae, Oxalidaceae, Geraniaceae¹ and many other families.

In the above-mentioned cases the cords which emerge on the petal radii are in no case simple midrib bundles. Their composition varies but is always complex; they are *trunk* cords. They may consist of (a) the marginal veins of the sepals and the petal midribs (Cruciferae, most Caryophyllaceae), (b) sepal marginal veins, petal midribs and superposed stamen midribs (many Primulaceae, few Caryophyllaceae, some Leguminosae), (c) sepal marginal veins, petal midribs, superposed stamen midribs, superposed carpel midribs (few Primulaceae), (d) petal midribs and superposed stamen midribs (many Primulaceae). A. Arber rejects this interpretation of the appearances in the Cruciferae on the ground that in some cruciferous types other vascular elements destined for the inner whorls occur in the central cylinder between those destined for the sepal midribs and those of the trunk cords on the alternate radii, thus precluding, in her view, the possibility of any lateral transference of elements from one to the other. But this argument does not take cognisance of all the facts. As the perianth bundles emerge it can be observed that they still show connections extending round to the inner face of the residual central strands (see Fig. 4). If such lateral transference as is envisaged depends upon continuity at this level, it is not precluded, though for that matter, it may conceivably occur below the level at which the midrib strand turns outwards. After comparing the various appearances in many types belonging to the above-mentioned families, one can have little doubt that the mode of origin of the sepal marginal veins is to be interpreted in the same way in all, and that the departure of these veins from the central cylinder separately from the sepal midribs is most naturally explained as the result of the elements from which these lateral veins are differentiated having become associated with those which differentiate into the midribs for the petals.

In the further course of her account of the vascular relations of sepals and petals, A. Arber, in support of her position, recalls that it was pointed out by Čelakovský(8) that the anatomical method carried to its extreme would lead to the *reductio ad absurdum* of regarding the marginal regions of the Crucifer sepals as of petal nature (4), p. 29). To this pronouncement it may be replied that

¹ As, however, in many Geraniaceae the trunk cords for the sepal marginal veins and petal midribs become dissociated into their components immediately on leaving the central cylinder the mode of origin of the former bundles is not nearly so obvious in this case as in the other families cited.



Figs. 8, 9. Leguminosae. Both from transverse sections. 8. *Astragalus hamosus* L. Flower base after the trunk cords for sepals, petals and stamens have left the central cylinder. Each of the three front trunk cords consists of the midrib bundles for a sepal, a neighbouring petal and the two superposed stamens and is becoming dissociated into its components. The trunk cords for the other perianth members have issued separately conjoined with the corresponding stamen bundles. 9. *Psoralea bituminosa* L. Flower base at the level at which perianth and stamen tube are becoming disjoined from the gynoeceum. The trunk cords which supply the sepal midribs and superposed stamens have become dissociated into their components; those on the alternate radii are in process of disjunction into two sepal marginal veins and a petal midrib bundle, or are already completely dissociated (see above and below on the right where the superposed stamen bundles have also now become detached).

pm, petal midrib; *sm*, sepal midrib; *smb*, sepal marginal bundle; *stb*, stamen bundle.

Čelakovský's verdict can be impugned; and further that the evidence afforded by the vascular anatomy of normal structures in regard to the relations of plant members is of the highest value (if only we can interpret it), for it reveals chapters in history which would not otherwise be plain to read.

In the Cruciferae, as described above, the sepal marginal veins are visualised as emerging from the central cylinder conjoined with the petal midribs. Later, after the sepal strands have become detached, the midrib bundles pass into the petals. Here then we should not expect the calyx to take on any marked corolla-like character, and none is apparent in those cases which have been examined. In contrast to the Cruciferae, let us take a case where the corresponding trunk cords do not become dissociated into three strands—two sepal marginal veins and a petal midrib, but divide simply in two, half going to the perianth member on either side. This condition is to be found in certain members of the Sterculiaceae. Now this family includes some genera with both calyx and corolla, and others having only a single perianth whorl. In these latter genera the *one* perianth whorl receives the *whole* of the outgoing bundles on *both* sets of radii, the one set furnishing the midribs, the other set the marginal veins. What is the result?

The result is clearly seen in such types as *Sterculia* (*Brachychiton*) *discolor* F. Muell. (Sterculiaceae) and *Rivina humilis* L. (Phytolaccaceae). In *Brachychiton*, where the lateral (= commissural) veins lie nearer the midrib than the margins, the thick tepal exhibits on its outer surface the character of a sepal, being greenish brown and hairy and on its inner face the appearance of a petal, being rose pink and smooth, while the induplicate margins are smooth and pink both inside and out (see (21), p. 100). In *Rivina*, in which the tepals are white, the marginal strips of the two outer members especially are slightly coloured. The perianth in this type is persistent and undergoes a significant change as the fruit develops. The marginal coloration disappears and the white ground gives place to green. That is to say, at the stage at which, in a type possessing both calyx and corolla, the petals would be withered or shed, the corolla character fades out in the *Rivina* tepals and the calyx influence thenceforward becomes predominant. Here we have two conspicuous instances showing that when the members of a single perianth receive commissural veins they exhibit characters which indicate that each individual tepal corresponds to a sepal upon which is superposed half the neighbouring petal on either side. Thus it is to be observed that the supposed

absurdity is a demonstrable fact. For the two cases cited above are not isolated examples of this relation. It may indeed be said that the old problem as to whether the tepal should be regarded as corresponding to a sepal or a petal now receives an answer. Owing to limitations of space, however, the whole of the evidence bearing on this question cannot be given here, but must be reserved for a separate communication.

Although it may be thought simpler in practice and hence preferable, despite the conclusions to be drawn from such facts as those given above, to retain existing terminology and to treat tepals of the above type as unit perianth members, we shall certainly not arrive at a full understanding of the interrelations of successive whorls unless the main features of the vascular anatomy with their implications are recognised and appreciated. In order to bear out this statement one needs only to recall the familiar case of the orchidaceous flower. Though we are in the habit of stating that in the typical Orchid flower the androecium consists of three members we recognise the fact that three additional vascular bundles are often to be found in the position corresponding to the three missing members, these bundles, in many cases, running in the labellum, the presence of the lateral pair being sometimes reflected in the outline of this structure. In this case the vascular anatomy provides the clue required to enable us to understand the relation of the Orchid ground plan to that of the typical Monocotyledon.

(b) *The androecium.* A. Arber's observations confirm the mode of origin of the bundles for the inner stamen whorl from four independent diagonal vascular strands, as shown in my earlier account (see (10), p. 124, Fig. 5, and p. 133), in which I have pointed out that *A4* and not *A2* expresses the true relations of the inner stamens.

(c) *The gynoecium.* The sections in A. Arber's accounts dealing with the Crucifer gynoecium reveal a curious attitude. This writer states that "to ask how many carpels are involved in such a gynaeceum is a purely scholastic question which can never receive an answer, because no answer exists" ((4), p. 39). At the same time she considers that the carpel conception should be retained for the purposes of description, but argues that the bicarpellary rather than the quadricarpellary theory should be accepted ((4), p. 40). In the course of this argument this writer sets out her grounds for rejecting the theory of Carpel Polymorphism. It is perhaps not surprising, in view of this writer's attitude, as indicated above, that the exposition of the theory should show a serious misconception of its implications,

resulting inevitably in misrepresentation, and further in unwarranted assertions. For this reason it is well at the outset to state definitely *that not a single piece of evidence brought forward by this writer controverts the theory of Carpel Polymorphism*, notwithstanding her assertion that "the anatomical basis on which the quadricarpellary theory rests [i.e. in the Cruciferae] does not stand examination" (4), p. 38). That the above italicised statement is justified will be clear from the following consideration of the various points raised.

In the first place one finds in A. Arber's discussion of the gynoecium, as in her treatment of the shoot, that there is the apparent assumption that if the labels are altered the facts cease to be true. As I have pointed out, the principle of the Leaf-skin is not bound up with any particular view of the phylogenetic origin of the leaf. Similarly the principle underlying the theory of Carpel Polymorphism, viz. that the syncarpous gynoecium is composed of units of different kinds, would not cease to hold if, with increased knowledge, our notion of the precise nature of the units underwent a change, making the use of some other term for these components desirable. It would still remain a fact that in the typical Crucifer gynoecium four components are recognisable, each with its own vascular system¹, and that these components constitute two pairs which differ in form and function. The ground plan would consequently still be represented by G₄, whatever hypothetical precursor or derivative be substituted for the carpel envisaged by the theory of Carpel Polymorphism. At present, however, it may be said that there seems no more reason for suggesting that the syncarpous gynoecium is not composed of the same order of unit as the apocarpous gynoecium, than for suggesting that the gamopetalous corolla is not composed of the same units as the polypetalous corolla.

In the section devoted to a consideration of the vascular anatomy of the gynoecium and its bearing on the theory of Carpel Polymorphism we find the following statement: "The assumption that the number of vascular bundles in the base of the ovary is an *absolute* guide to the number of carpels, is treated as axiomatic, and *no* proof of it is offered; it is even referred to as the 'one cord to each carpel' rule" (4), p. 37)². To this assertion it may be replied that, as has been previously set forth in detail, the sum total of *all* the characters in the Cruciferae, *including the fact that the ovary commonly*

¹ These systems being entirely distinct in their origin though anastomosing eventually.

² It is perhaps unnecessary to point out the exaggerated character of both statements, though the italics are mine.

these four orthogonal main vascular cords (Figs. 16, 17, 21), are in harmony with the conception that the gynoecium is tetramerous, whereas on the view that only two carpels are present, the vascular anatomy, stigma position, replum development, mode of dehiscence of the fruit, demarcation of the ovary surface in a manner in accordance with construction from four components, all constitute so many anomalies. It seems necessary to recall once again that there is no warrant for the old fiction that the replum is a double structure in relation to the median plane. This assumption has to be made, however, on the bicarpellary interpretation, and A. Arber makes it lightly enough. For the early supporters of the bicarpellary view at least imagined that they had grounds for their assumption in the appearance of the two sheets of superficial cells, which form two visibly separate layers if the intervening tissue is scanty or becomes torn or dried up as the fruit develops. But with the removal of this misconception no real justification for this assumption exists.

If the whole gynoecium of such types as *Cheiranthus* or *Matthiola* are examined at a very early stage in differentiation of the vascular tissue four strands are seen passing upwards about equally distant from one another. Differentiation may proceed at different rates in the two pairs (median and lateral). Thus in a very young bud of *Cheiranthus Cheiri* L. at the stage shown in Fig. 10, the differentiated median (replum) strands have already made their way more than half way up the ovary while there is, as yet, no trace of differentiation of the lateral (valve) pair. In this species the median strands keep their lead throughout, and it is therefore not surprising to find that the stigma lobes, which are here distinct, are centred over these cords, for the lateral (valve) strands come to an end at the base of the short style. In *Matthiola incana* B.Rr. (Figs. 11-15), on the other hand, the valve strands develop at first more rapidly than the median pair (Fig. 11), but this lag is shortly made good, all four strands reaching the stigma level (Fig. 12). As development proceeds the valve strands remain for the most part unbranched in both these types (Figs. 13, 14, 22), but the median strands branch in a typically pinnate manner beginning at the base and continuing up the whole length of the ovary (Figs. 15, 23). These branches turn out horizontally and curving round run for a short distance in the outer wall of the loculus. In the meantime the main strand breaks up into two chief branches which run upwards in the replum. It is with these longitudinal strands that the funicle bundles eventually make connection, though not until very much later. It is impossible to conceive of this whole system as

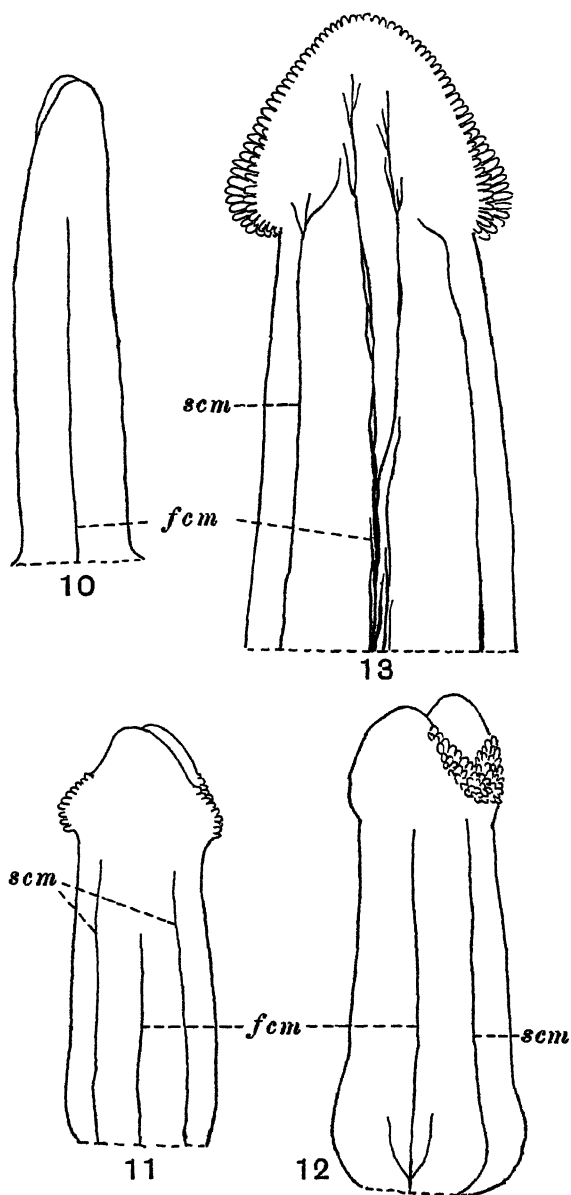
arising from the fusion of two marginal veins. The rational interpretation of such a system is that it is what it looks to be—the system of a whole foliar member, a carpel. It may be added that the supposition that in those Crucifer types in which the replum comes to have twin bundles these bundles together constitute a carpel midrib is not without parallel elsewhere. We meet with precisely the same arrangement in some Leguminosae (species of *Astragalus*) (20), p. 250, Figs. 70, 73, 74).

Again, if we take the case of *Bunias* (Figs. 16–20), we find an even greater difficulty in attempting to reconcile the facts with a dimerous ground plan. Here the ovary is four-sided. Each quadrant has a main bundle with a pair of lateral veins, the four systems being in this respect precisely similar. (The main bundles in the two median quadrants also give off towards the centre the branches with which the funicle strands connect.) I have previously emphasised the clear nature of the evidence for tetramery in *Bunias* (17, 19), yet in her account of the few selected types with which she deals, A. Arber leaves all such difficulties as those here described untouched.

It seems well at this point to insist that no view of the constitution of the siliqua which is not found to be equally in accord with the facts in allied families can be accepted. When, however, at the outset of the present investigation observation was extended to other nearly related families it became apparent that the theory of Carpel Polymorphism, *including the conception that each carpel receives a single main vascular cord which furnishes the midrib*, provided an explanation of the characters of the gynoeceium both in normal and anomalous flowers. As observation was further extended to family after family among both Dicotyledons and Monocotyledons, the same fundamental conception of the character, anatomical and morphological,

Figs. 10–13. Cruciferae. The gynoeceium seen in surface view after being rendered transparent. 10. *Cheiranthus Cheiri* L. A very young gynoeceium viewed from the front. In the mid-line a fertile carpel (replum) bundle. [At this stage the valve carpel bundles are not yet differentiated] 11–13. *Matthiola incana* R. Br. 11. A gynoeceium slightly older than that shown in 10 viewed from the front, showing a fertile carpel midrib in the centre and a valve carpel midrib on either side. All three cords are unbranched. Towards the top the stigmatic papillae centred over the valves but not yet extending over the central cone. 12. A slightly older gynoeceium turned slightly sideways. Near the centre a fertile carpel bundle with two branches, on the right a valve carpel midrib, unbranched. Stigmatic papillae extend further up the conical apex than in 11. 13. Upper half of an older gynoeceium viewed from the front, showing further development of the sterile and fertile carpel bundles. Stigmatic papillae now cover the whole apex.

*fc*m, fertile carpel midrib; *sc*m, sterile carpel midrib.



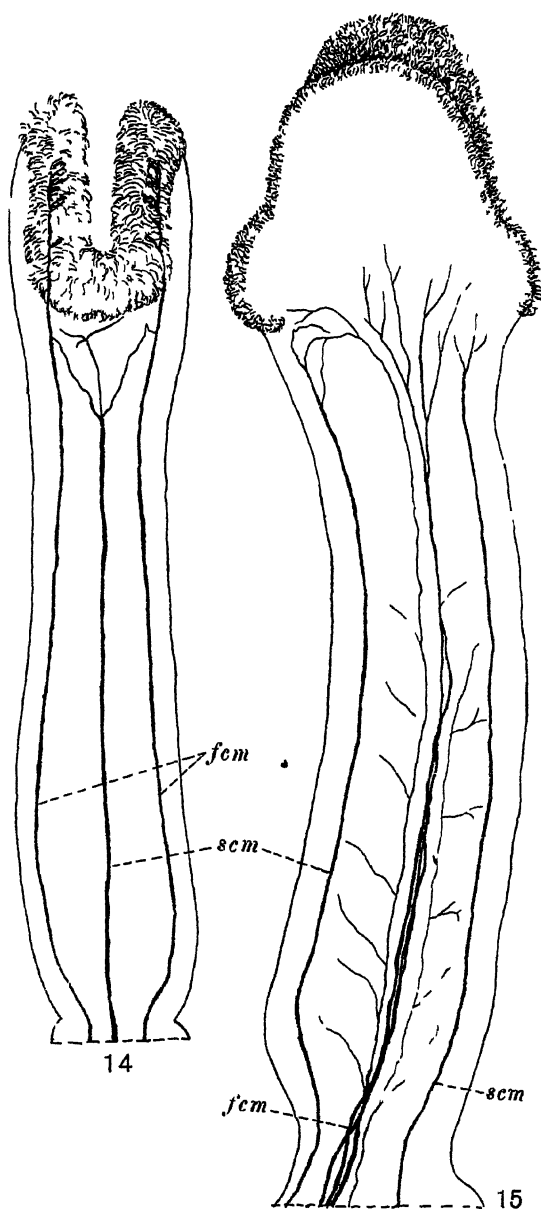
Figs. 10-13

of the carpels was found to fit the observed facts. In these circumstances "one cord to each carpel" may fitly be termed a rule, and, as a rule deduced from observation in a wide field, there is reason to anticipate that it will be found to hold throughout the range of flowering plants.

One of the facts which A. Arber cites in support of her assertion that the anatomical basis on which the quadricarpellary interpretation rests "does not stand examination" is that the four bundles which are left in many Crucifers after the exit of the bundles for the inner stamens are not always identical with the four bundles seen at a higher level which, on the above interpretation, constitute the midrib bundles of four carpels (4), pp. 37, 38). This argument is curiously illogical, coming from this writer. Since A. Arber adopts the bicarpellary interpretation it is clear that she recognises that it is not necessary that the bundles left in the centre immediately after the outward passage of the bundles for the inner stamens should be identical with the carpel midrib bundles. On the bicarpellary view the four bundles in question *eventually* become differentiated into two midribs; on the quadricarpellary view they *eventually* become differentiated into four midribs. But if, on the former view, the residual bundles are not considered to be carpel midribs until the level at which the process of reorganisation is complete, the same criterion must also be applicable on the latter view. Apart, however, from the illogical nature of this writer's argument, it is to be noted that the assumption on which it is based is altogether erroneous. It wholly ignores the fact that reorganisation of the residual vascular tissue takes place between the origin of each floral whorl, though the successive whorls ordinarily follow so closely that distinct internodes are not appreciable. Although we are unable to analyse the steps in this reconstitution process we must recognise that it takes place. A further circumstance which invalidates the argument is the fact that in many Cruciferae (as described later) undifferentiated strands

Figs. 14, 15. Cruciferae. *Matthiola incana* R.Br. (continued). 14. Gynoecium of open but unfertilised flower viewed from the side after being rendered transparent. The valve carpel midrib remains unbranched until near the stigma level. 15. A similar gynoecium from another flower split in half in the lateral plane and similarly treated, viewed from inside. In the centre a fertile carpel midrib bundle which gives off horizontal branches which run in the ovary wall, and longitudinal branches, to which the funicle strands become connected later. To right and left the valve carpel midribs which remain unbranched until the stigma level is approached. [For the sake of simplicity the loculi and ovules are omitted.]

fcm, fertile carpel midrib; *scm*, sterile carpel midrib.



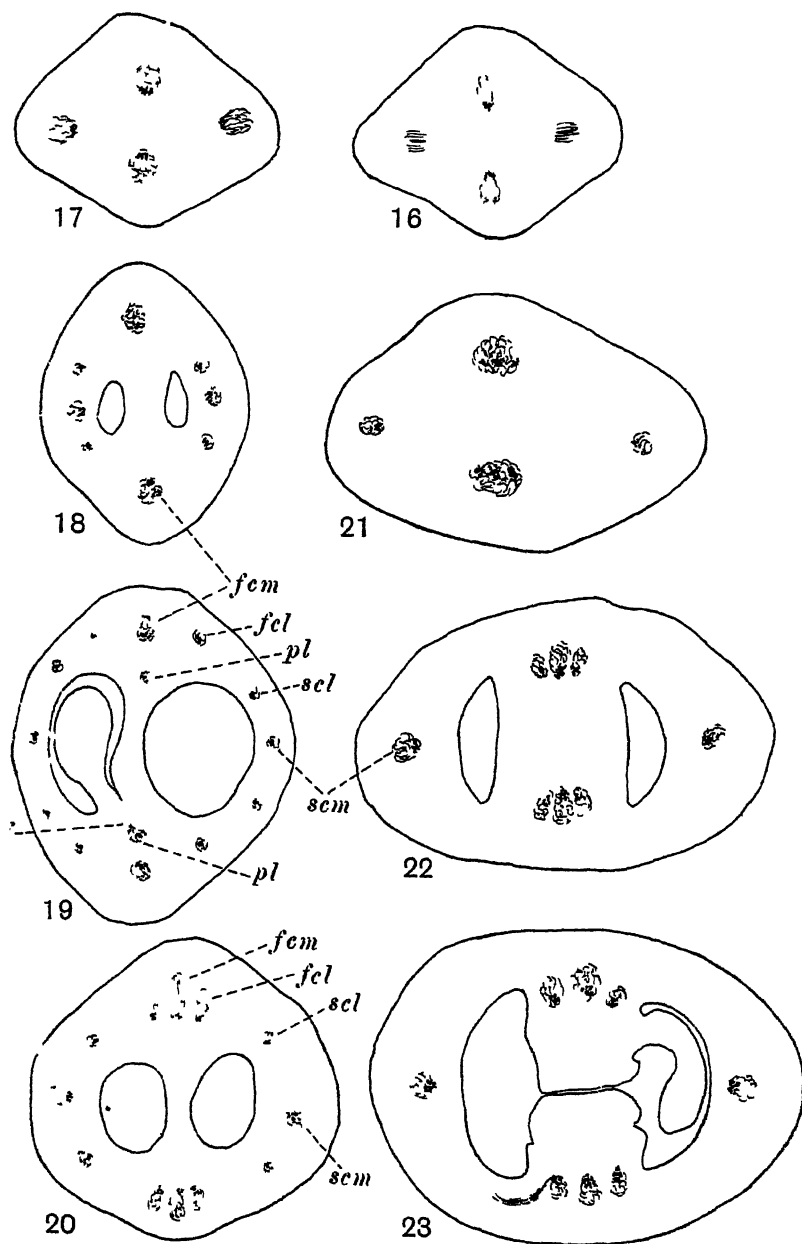
Figs. 14, 15.

occur at the level in question on the radii between the differentiated cords (Figs. 2, 3) and either cease later or merge with these cords so that they are no longer traceable, thus rendering it still further evident that the recognisable vascular units at this level will not necessarily be identical with those at a higher level.

This writer further argues (*loc. cit.*) that the fact that the narrow siliqua of the typical Crucifer when young is similar in form to the silicula of *Lunaria* tells more in favour of the bicarpellary than of the quadricarpellary interpretation of the gynoecium. For on the former interpretation the fruit valves of the siliqua represent the two lateral valve carpels, whereas, taking the limits of the venation system to indicate the carpel limits, I have suggested that in *Lunaria* each fruit valve consists of a lateral valve carpel + half the lamina portion of each of the two median semi-solid carpels. As a purely abstract argument, A. Arber's contention may seem to have some validity, but the consideration of certain facts shows that little weight, if any, can be attached to it. Let us take another case for comparison. The young leguminous ovary is of much the same form in most types. Nevertheless the fruit valves are not always equivalent structures, and this remains true whether one accepts a bicarpellary interpretation or adheres to the monocarpellary view. In the ordinary case dehiscence takes place in the median plane; in the *Haematoxylon* type the fruit tears in the lateral plane; in *Carmichaelia* we meet with the unusual condition in which the two fruit valves consist of the carpel laminae only, the bundles back and front both remaining behind on the fruit stalk; in the coiled pod of *Medicago* splitting occurs close to, and on each side of, the front midrib bundle which comes away whole

Figs. 16-23. Cruciferae (continued). All from transverse sections taken when in series from below upwards. 16-19. *Bunias Erucago* L. 16. Ovary base with four orthogonal vascular bundles, the two in the lateral plane cut obliquely as they turn outwards. 17. The same after the lateral bundles have turned upwards. 18. The same after the appearance of two loculi. The lateral carpel bundles have given off a pair of lateral veins. 19. The ovary in the ovule-bearing region. The median carpel bundles have formed two lateral veins similar to those of the lateral carpels and in addition the two placental bundles. 20. *Bunias orientalis* L. The ovary at a level between that shown in 18 and that of 19. Both lateral and median carpels have given off a pair of lateral veins but the placental strands are not yet formed. 21-23. *Matthiola incana* R.Br. 21. Ovary base showing four orthogonal vascular bundles. 22, 23. The same after the appearance of the loculi and the formation of lateral veins by the median carpel bundles. Below on the left in 23, a branch from one of these veins is seen cut longitudinally as it curves round into the wall of the loculus.

f, funicle strand; *fcl*, fertile carpel lateral bundle; *fcm*, fertile carpel midrib; *pl*, placental bundle; *scl*, sterile carpel lateral bundle; *scm*, sterile carpel midrib.



Figs 16-23.

from the rest of the fruit. It will thus be seen that the fruit valves have a different composition in each of these four types. But what occurs in the Leguminosae is not ruled out in the Cruciferae. Whether fruit dehiscence occurs at the carpel boundaries or elsewhere depends upon the distribution and character of the tissues and upon the tensions consequently set up. In the typical legume splitting takes place in the mid-line back and front owing to the drying up of the parenchymatous tissue and the cracking, in the case of the front midrib, of the surrounding arc of sclerenchyma. In *Carmichaelia* the two cords become embedded in a solid mass of sclerenchyma, which prevents dehiscence in the median plane either back or front. Here the secondary veins are so few and weak that as the rest of the tissue dries they readily snap and allow the valves to fall. In *Medicago* the weak lateral veins of the front midrib are also easily snapped. In the *Haematoxylon* type the nature and distribution of the tissues again prevents dehiscence in the median plane, and irregular tearing occurs down the flat sides of the pod, in the region where the two vascular systems come to an end. Thus in certain Leguminosae the distribution of the tissues is such as to cause dehiscence to occur in planes which cut across the plane in which the meeting edges of the carpels lie¹. Similar variations occur in the Cruciferae. In the typical siliqua dehiscence takes place in the region where the four vascular systems meet and anastomose, because here also is situated the mechanical tissue which, in this case, is the effective agent in bringing about dehiscence. In *Lunaria* dehiscence is brought about in the same way, but here the position of the mechanical tissue does not coincide with these boundaries, hence the planes of dehiscence cut across the planes in which the carpel boundaries lie. It will thus be seen that it does not follow that the fruit valves of types belonging to the same family, having ovaries of the same number of carpels, and of the same form when young, will necessarily have what, for brevity, may be termed the same carpel equivalence, hence A. Arber's argument becomes groundless.

At a later point (5), pp. 198, 199), A. Arber discusses certain variations from the typical Crucifer gynoeceium under the head of teratology and atavism, dealing more particularly with *Matthiola* and *Capsella I'guieri* Blar. The refutation, point by point, of the arguments advanced would necessitate the verbatim citation of this whole section, which is impracticable. It must suffice to state in general that the whole line of reasoning is as unsound here as I have

¹ For a fuller account of the ovary of the Leguminosae see (20).

shown it to be in the points already considered, and to treat in detail the most important of the issues raised.

A study of meristic variations shows that this class of deviation from the type can by no means be referred to a single category(7). The plausible statement that any sporadic variation of this kind might equally well be called futuristic as atavistic is only partly true, if true at all. The study of heredity reveals that in some cases the sporadic appearance of a variation in structure undoubtedly takes place because such a structure was a characteristic of the race in the past. If, reappearing in a mutant, it should persist in this individual's descendants, then at its first reappearance it might, to use this writer's terminology, be described as futuristic, *but it would be so because it is also atavistic*. We have good grounds for holding that some deviations from the type have an atavistic significance and that others have not. For example, the appearance of a horned calf in a breed of polled cattle would have a different significance from that attaching to the appearance of a two-headed calf in the same breed. Yet this writer maintains that if the four-valved ovaries of *Matthiola* are regarded as atavistic then, since she has observed the occurrence of accessory buds in the axils of the petals of the lower flowers of *Nasturtium officinale* R.Br., it would be equally legitimate to describe this latter deviation as reversionary, which reasoning, she argues, leads again to a *reductio ad absurdum*. But because two quite different variations happen to occur in the same region in any species, it does not necessarily follow that the two mutations have the same significance. To continue the illustration given above, if the two-headed calf happened to develop horns we should rightly attribute an atavistic significance to the one exceptional feature but not to the other. Even apart from this all-important distinction, the illustration of *Nasturtium* is a particularly bad one upon which to rest the argument in support of which it is cited. Our knowledge of the relations of stem and leaf leads us to believe that the Dicotyledon plant has the potential capacity to develop a bud in association with every leaf member, though this potentiality is almost always dormant in the flower. The development of buds in the axils of floral leaves, though not of frequent occurrence, has been observed from time to time in various genera. It is characteristic of some double-flowered strains of *Prunus*. The appearance of these buds is the result of the prolongation of an inherent activity which usually ceases at an earlier stage in development. We are obviously not dealing here with a *new* character, but with the recrudescence of an activity manifested in youth.

Nor is this writer's argument any better founded in regard to *Capsella Viguieri* in which the majority, but not all, of the ovaries are four-valved and the axes often, but not invariably, fasciated. My suggestion that the four-valved condition may be atavistic here, as well as in the Cruciferae generally, finds support in certain facts of vascular anatomy. I know of no evidence (as distinct from argument) that could possibly be supposed to support a similar interpretation of the fasciated condition.

In many cruciferous types the residual central cylinder shows, as I have stated above (pp. 193-4), eight groups of vascular elements in a cross-section taken at about the level at which the outer whorls become exserted. Of these eight groups, four stand in the orthogonal planes and already, before the bud expands, show a well-developed xylem. The other four occur in the intervening spaces and are undifferentiated. The four differentiated strands undergo further differentiation into the four main cords which, on the quadricarpellary view, become the midribs of four orthogonal carpels. The four undifferentiated strands shortly cease to be traceable, either coming to an end or merging with the alternate strands. Here, as in a large number of Dicotyledons, we see superfluous vascular elements remaining over after those destined for the last existing whorl have been organised into the corresponding midrib bundles. There is general acceptance of the view that reduction has taken place very widely throughout the flowering plants in the number of the members composing the whorls of the androecium and gynoecium. We find abundant evidence of this process in the presence of superfluous vascular strands in a position which shows that they belong to suppressed members of existing whorls. But, as in the above case of the Cruciferae, we also find residual vascular elements in the position which suggests that they are the remnants of bundles which once served a whole additional carpel whorl. Now in *Capsella Viguieri* the vascular strands lying between the four orthogonal bundles for the first four carpels, unlike those in most other cruciferous types, undergo differentiation and show a well-developed xylem. These intervening strands give rise to four diagonal bundles, and after these to a further set of four orthogonal bundles. That is to say, the vascular supply of the gynoecium is arranged as 4 orthogonal + 4 diagonal - 4 orthogonal bundles. Can one doubt that these bundles represent the midrib bundles of three carpel whorls, 4 orthogonal + 4 diagonal - 4 orthogonal? Of these three whorls the outer is sterile and of valve form, the middle whorl, consolidated and fertile, the third, also

probably consolidated, is sometimes fertile, though owing to lack of space this whorl is often more or less deformed and may fail to develop ovules. In her account of this form, A. Arber refers to the structure formed by this third whorl as "a curious structure" which "takes the form of a central lobe of tissue, which holds apart the four replums of the main ovary." "This central lobe," she adds, "bears ovules." Later, referring to the four bundles which supply the structure described as a "central lobe," she contends that these bundles are not *residual* but are derived by *branching* (my italics) from the bundles which supply the four repla (i.e. on the polymorphic view, the four members of the second carpel whorl). This account of the facts is as misleading as the earlier account dealing with the sepal marginal bundles (discussed above, p. 183) and with the normal ovary. The citation of a single sentence will suffice to make this clear. After tracing the development of the different bundles of the gynoeceium "practically element by element," this author finds that "the bundles of the lateral valves were derived entirely from the replum bundles." Here again, the successive steps in development are presented in reverse order. The bundles for a more central group of structures are described as giving off branches which become the midribs of an outer set of members. As to the value of a meticulous examination of the make-up of the vascular strands by tracing the mode of origin of one from another "element by element," I have grave doubts. The original relative position of these elements has long previously been disturbed by the process of "sliding growth" accompanying development. If any significance in the elucidation of the development of plant members attaches to these space relations of the individual vascular elements, then these relations will presumably have to be traced back as far as the meristem. But if we turn from the method of investigation to the facts described, what general picture do we obtain of the processes underlying the orderly and regular results of development? Conceivably there was no intention to go beyond the bare description. Certainly no attempt is made to explain the nature of the so-called "central lobe." Let us then review the facts in their proper developmental sequence. After the exit of the four bundles for the sterile values there remain behind in the centre, not simple replum bundles, but residual strands. These residual strands are reorganised. The result of this reorganisation process, a process analogous with that taking place at the nodes of the vegetative axis but here effected more simply, is that four equivalent bundles first come into being, which regularly take up their position in the diagonal planes and supply the

ovules present in the outer ovary; and later, four similar bundles disposed in the alternate (orthogonal) planes which supply the ovules developed within a second inner ovary. The fact that the elements for this last set of four strands are at first associated with the preceding set of four strands and later become detached does not affect their residual character. They are residual in the sense that they are left over after differentiation of the preceding whorl. This kind of association is of widespread occurrence. In many types, notably in various species of *Primula*, it is characteristic for the bundles destined for the carpels to be conjoined at first with the corolla-stamen cords, and, as these trunk cords become dissociated, to turn inwards and take up their proper position. But such bundles are just as properly designated residual bundles, which in due course become differentiated into midribs, as those which in other *Primula* species are left behind in the centre when the perianth bundles turn outwards¹. The same remark applies to the case of *Capsella Viguieri*. In this latter type the dissociated strands in question take up the position proper to a third carpel whorl; the structure which they supply may develop a cavity through the cessation of the pith as in any typical syncarpous ovary; the cavity may contain ovules; the structure itself or its separate components may be terminated by stigmatic papillae. It is difficult to imagine what further evidence is required to demonstrate the carpel nature of the components of this terminal body, which stands erect and free within the cavity of the outer ovary. The term "lobe" which would hardly seem applicable as a description, let us say, of the central, erect, ovule-bearing column in the Primulaceae, is scarcely more happily used here to indicate the apex of the axis with its last whorl of ovule-bearing appendages.

* Another statement in regard to *C. Viguieri* runs as follows (5), p. 177): "On the quadricarpellary view the passage from one type to another (i.e. from *Capsella Bursa-pastoris* to *C. Viguieri*) becomes a highly complex matter, since this view interprets each replum as a single complete carpel. The *Viguieri* form cannot on this view be reached from the *Bursa-pastoris* form without a change more fundamental than mere addition of carpels—namely their re-orientation" (the italics are mine). This assertion shows a fundamental misconception of the application of the theory of Carpel Polymorphism to the case of the Cruciferae, for the statement is wholly devoid of truth. Now the suggested re-orientation of carpels supposes that those

¹ These two modes of differentiation in *Primula* are fully described and figured in my account of the Primulaceae (see (23)).

occupying a certain position in the one *Capsella* form are situated in a different position in the other form. As long ago as 1926 I pointed out that the traditional interpretation requires the assumption of re-orientation of the carpels in the case of two species of *Reseda*, a difficulty completely avoided on the theory of Carpel Polymorphism, which offers a rational solution of the two ground plans, without any such assumption. On the former view that only three carpels are present in either species, it has to be supposed that the three carpels occupy one set of radii in the one case and the alternate set in the other case, *R. odorata* L. having, on this view, one posterior and two antero-lateral, *R. luteola* L., one anterior and two postero-lateral valve carpels (see (16), pp. 302-4 and Figs. 3, 4). *On the theory of Carpel Polymorphism no such re-orientation needs to be postulated.* On this latter theory six carpels are present in both types, disposed on identical radii, three being fertile and three sterile, but those that are fertile in the one form are sterile in the other, and *vice versa*. In the two forms *Capsella Bursa-pastoris* and *C. Viguieri* we have another instance of a change of rôle among the carpels, *but there is no re-orientation.* In *C. Bursa-pastoris* the gynoeceum ground plan is represented by a single whorl of four orthogonal carpels; that of a typical flower of *C. Viguieri* similarly shows a first whorl of four orthogonal carpels; the succeeding whorl is naturally diagonal, and if a complete third whorl is present this whorl is naturally orthogonal. The two forms thus differ, *not in the orientation of the corresponding whorls but in the rôles which the carpels play.* In *C. Bursa-pastoris* the two median carpels are consolidated and fertile, the two lateral valve-like and sterile. In *C. Viguieri* all four members of the corresponding (outermost) whorl are valve-like and sterile; those of the next whorl are consolidated and fertile, as also are those of the final whorl. That carpels can, and do, undergo change of form is clearly shown in another case. This same plastic quality is seen in another mutant of *C. Bursa-pastoris*, viz. *C. Heegeri* Solms. *C. Heegeri* differs from the type solely in the top-shaped form of the ovary, this change in shape, unexplained on the bicarpellary interpretation, being attributed on the quadricarpellary view to the lateral valve carpels having become somewhat extended, and the median consolidated carpels somewhat contracted. Yet another instance of the same kind of phenomenon is occasionally met with in another cruciferous genus, *Biscutella*, when one of the two valve carpels fails to expand and takes on the consolidated form ((17), p. 49, Figs. 11-21). Ignoring these facts, A. Arber claims that a simpler explanation of *C. Viguieri*

is to presume that two new carpels have sprung into existence, not forming an additional whorl but intercalated between those ordinarily present. This assumption is unsupported by any evidence. Any puzzle can be *simply* solved by making the assumption which gives the required answer, but the validity of the assumption and not mere simplicity is the test of its acceptableness.

Before leaving this subject it seems well to recall that we have long been familiar with a similar interchange of rôles among members of the *androecium*. In the ordinary orchidaceous type we find one (originally) anterior stamen and in many cases two antero-lateral staminodes, but in *Cypripedium* and its allies the anterior member appears as a staminode and the two antero-lateral members are fertile. There is no ground for supposing that a similar reversal of function may not take place among members of the *gynoecium*.

Yet a further remark of this writer in regard to *C. Viguieri* calls for passing comment. It is stated that "the form (*C. Viguieri*) has proved constant under cultivation during the period of twenty years that has elapsed" (i.e. since the original individual was discovered) (5), p. 173). No breeding data are cited in support of this assertion. The reader will naturally take the above statement to refer to the two characters, fasciation of the stem and the four-valved form of ovary. But as I stated earlier (after having bred this form) neither character is constant (1b), p. 187). Fasciation does not occur in all plants, and some individuals produce a variable number of three- and two-valved fruits besides the four-valved type. In respect of the ovary character, *C. Viguieri* bears to *C. Bursa-pastoris* the same relation that *Tetrapoma* bears to a species of *Nasturtium* and *Holargidium* to a species of *Draba*. As I have previously pointed out (*loc. cit.*, p. 188), the fundamental distinction between *C. Viguieri* and *C. Bursa-pastoris* lies in the proliferation of the axis of the former type, and in the formation of additional carpel whorls, the whorls being composed in the majority of the flowers of four carpels, but in a minority of three or two carpels.

Finally it remains to refute in the most emphatic terms compatible with the courtesies of controversy the implication contained in the following statement with which A. Arber concludes her comments on my interpretation of the siliqua and the latiseptal silicula, based on the theory of Carpel Polymorphism. "It is probable," she writes, "that these different explanations would never have been proposed if attention had been primarily paid to prefertilisation stages" (4), p. 38). My reply to this opinion, previously expressed

verbally by this writer at the Fifth International Botanical Congress held at Cambridge in August, 1930, was given at the time ((22), p. 300). It may again be explicitly stated here that *throughout the series of investigations on Carpel Polymorphism material in the prefertilisation stages has invariably been employed*. This has been supplemented in those cases where it appeared desirable by the examination of a continuous series of stages both leading up to, and succeeding, fertilisation. In short; no greater travesty of the actual situation can be imagined than that contained in the passage cited above. It would appear that this writer was not aware at the above date in 1930 that it had already been suggested by other critics that I had reached a certain view of the carpel (rejected by them) through having directed my attention to *too early* stages in development. In the course of their remarks on the attachment of the ovules in the Cruciferae Eames and Wilson complain that they find in my account what they regard as a very inadequate discussion of this point and conclude that "the reason is doubtless that the actual attachment was not often seen" (an opinion as wholly contrary to the facts as A. Arber's cited above), "for," as they add, "Miss Saunders speaks of 'sections of buds and flowers'" (the italics are mine) ((10), pp. 647, 648). When critics are in such diametrical disagreement the dispassionate reader will perhaps be disposed to discount their criticism and to acquit the accused.

It remains to deal with the position of the cruciferous stigma. The commissural stigma is an anomaly which is not confined to this one family: it is met with in individual genera in many families, and throughout in others. It is, nevertheless, an exceptional feature and, as such, calls for explanation. A. Arber, confining her observations to the one family of the Cruciferae, suggests that when stigmas are commissural the carpel margins may be imagined to have been "promoted" to a "new status." This suggestion is backed by special pleading, by appeal to authority, and by the further suggestion that those types in this family such as *Matthiola* which have been regarded as having non-commissural stigmas have, in fact, been wrongly described. With regard to this last point it may be said that the case of *Matthiola incana* R.Br. is not altogether easy to decide, owing to the fact that the stigmas are sessile, but that the evidence, on the whole, supports the current description. In young *Matthiola* ovaries the four midrib bundles of sterile and fertile carpels (I use the terminology of the theory of Carpel Polymorphism) extend to about the same height (Fig. 12). The valve carpel midribs remain un-

branched until the stigma level is reached, when the four cords become connected by anastomoses arising from each (Figs. 13-15). At an older stage the fertile cords overtop the sterile bundles. The two lobes which surmount the gynoeceium stand in the median plane as in the case of *Cheiranthus*, where, however, the stigmatic areas are centred on these lobes. In *Cheiranthus* the valve carpel midribs end below the stigma, either blindly or in contact with the edge of the massive cord of one or other fertile carpel at the level of origin of the short style. On the other hand in *Matthiola*, as stated, both sterile and fertile cords extend to the stigma level, *the stigmatic papillae, however, are developed first over the ends of the valve carpel bundles* (Fig. 11) and from this region gradually extend up the sides of the two lobes formed by the somewhat taller fertile carpels (Fig. 12) until they become continuous over the whole apex of the gynoeceium (Figs. 13-15). But there is no separate focus of origin of the papillae in the mid-line of these lobes. The stigmatic papillae are then clearly *centred* over the valve carpels though they extend over the apices of the fertile members. If we turn from the Cruciferae to the Papaveraceae we find that although the stigmas are commissural in the great majority of genera, they are clearly centred on the alternate radii in *Platystemon*, *Hesperomecon linearis* Benth. and (so far as appears) in *Meconella californica* Torr., while in some of the Fumariaceae stigma structures appear to occupy both positions. Presumably here also, A. Arber would have recourse to the explanation that the ovule-bearing margins must be supposed to have attained to the "new status" in most types, although not in others. But on this view how is *Eschscholzia* to be accounted for, with stigmas varying in number from 2 to 20 in a gynoeceium with only the same number of ovule-bearing margins as in the Cruciferae? And what is to be said of those types scattered through different families in which the number of styles and stigmas is constantly twice as many as the number of carpels supposed to be present on the old conception that all carpels are monomorphic? Or of those in which there is occasionally an additional style and stigma without a corresponding loculus? The only uniform and simple interpretation of all these cases is that afforded by the theory of Carpel Polymorphism. On this theory commissural stigmas are not a reality, all stigmas so described being, in fact, centred over the midribs of carpels of the consolidated type.

Fumariaceae—Fumarioideae

(1) *The axis of the inflorescence.* In the course of her account of *Corydalis nobilis* Pers. A. Arber describes the last bract of the flowering shoot as arising from "a transformation of the entire shoot axis" and as being "strictly terminal" to that axis. This interpretation of the appearance is open to the same objection as the similar case previously instanced by this writer of the flowering glume in certain species of bamboo, for the *Corydalis* bract appears in cross-section as a complete ring at its base (see (6), p. 341, Fig. 13, A₄). Comparison of this writer's figures of *C. nobilis* with those of the corresponding region of *C. bulbosa* Reichb. (see (6), p. 327, Fig. 5, B₃, B₄), leaves it scarcely doubtful that the ring seen in the former species would not be complete if the termination of the non-vascular apex of the axis were not incorporated with it. In the case of *C. bulbosa* this ring is described as composed of bract together with non-vascular axis tissue ((6), Fig. 5, B₃), yet in *C. nobilis* this ring and the solid region behind it is interpreted as consisting of axis only, although a bract has yet to take shape. Axis and bract show no demarcation at the ring level in *C. bulbosa*; the axis only eventually becomes delimited because a remnant of tissue persists to the level at which the bract takes on leaf form and this remnant remains adherent to one margin. Now if this small remnant became somewhat smaller still, so that it disappeared altogether just at the level at which the ring broke and the bract took on its proper leaf form, we should expect to see exactly what is represented in (6), Fig. 13, A₄ and A₅. But in such case it would *not* be a fact that the axis apex was transformed into a leaf member or that the leaf member was "strictly terminal" to the axis. The whole question turns no doubt on definition. I submit that what is intended to be connoted by the general statement that an axis does not terminate in a leaf is the fact that a leaf does not arise from the meristem cells in the middle line of the axis apex, and that such pseudo- or quasi-terminal leaf members as those seen in bamboo and *Corydalis* do not invalidate this statement. And, furthermore, that nothing is gained by a mode of description which ignores this distinction between stem and leaf development. It may be added that it is not uncommon to find in a large genus considerable variation in the amount of the non-vascular prolongation of the flowering axis above the level of exsertion of the last leaf (carpel) whorl as is well illustrated in the case of *Hypericum*. It is therefore easily conceivable in cases where the last leaf member

is a small solitary structure, as in the case of a minute bract, and where no bud develops in its axil, that the persisting remnant of axis tissue should thin out to so small an amount that if it is not delimited from this bract its actual ending becomes imperceptible.

(2) *The androecium*. A. Arber holds that the four inner stamens with half anthers characterising the section Fumarioideae (*Dicentra*, *Corydalis*, *Fumaria*) should be regarded as complete stamens which have undergone reduction, so that the ground plan in this section should be represented by $A_2 + 4$, not by $A_2 + 2$ with the second, inner whorl represented by four half stamens. This writer rejects the suggestion made in my account of the vascular anatomy of these types, that the vascular material proper to two median stamens has undergone congenital division, the two products of division making their way out from the central ring, not in the median line where the petal midrib blocks the way, but to one side, where they have an unbarred passage (18), pp. 186-7). In support of her view she goes so far afield as the Cruciferae in order to produce an instance (*Atelanthera perpusilla* Hook. f. and Thoms.) where four undoubtedly whole stamens habitually suffer degeneration of two pollen sacs, *but*, it is to be noted, in a quite irregular manner, the suppressed sacs being apparently most frequently both those on the same side, more rarely one on each side of the connective. Now this scheme, put forward for one section of the Fumariaceae, is opposed to the evidence afforded by the other section of the family—the Hypecoideae, and, indeed, by certain abnormal flowers even among the Fumarioideae. No mention is made of these awkward facts. By treating the Fumarioideae separately, apart from the Hypecoideae, this writer has presented a one-sided picture of the whole androecium problem in this family. Since the Hypecoideae provide a serious objection to the view that the inner stamen whorl of the Fumarioideae consists of four diagonal, whole stamens with partially aborted anthers, it is not surprising that this writer has to rest her case again on special pleading and on a far-fetched comparison drawn from another family. In these circumstances it will be well briefly to review the whole position in the Fumariaceae.

Since in the section Fumarioideae the whole of the vascular supply of the sepal, petal and outer stamen whorls leaves the central cylinder in the form of four orthogonal trunk cords, it would not be wholly without parallel if these cords, although constituting two pairs, were to be followed by a single whorl of four cords for four whole members in the diagonal planes, for, as I have elsewhere

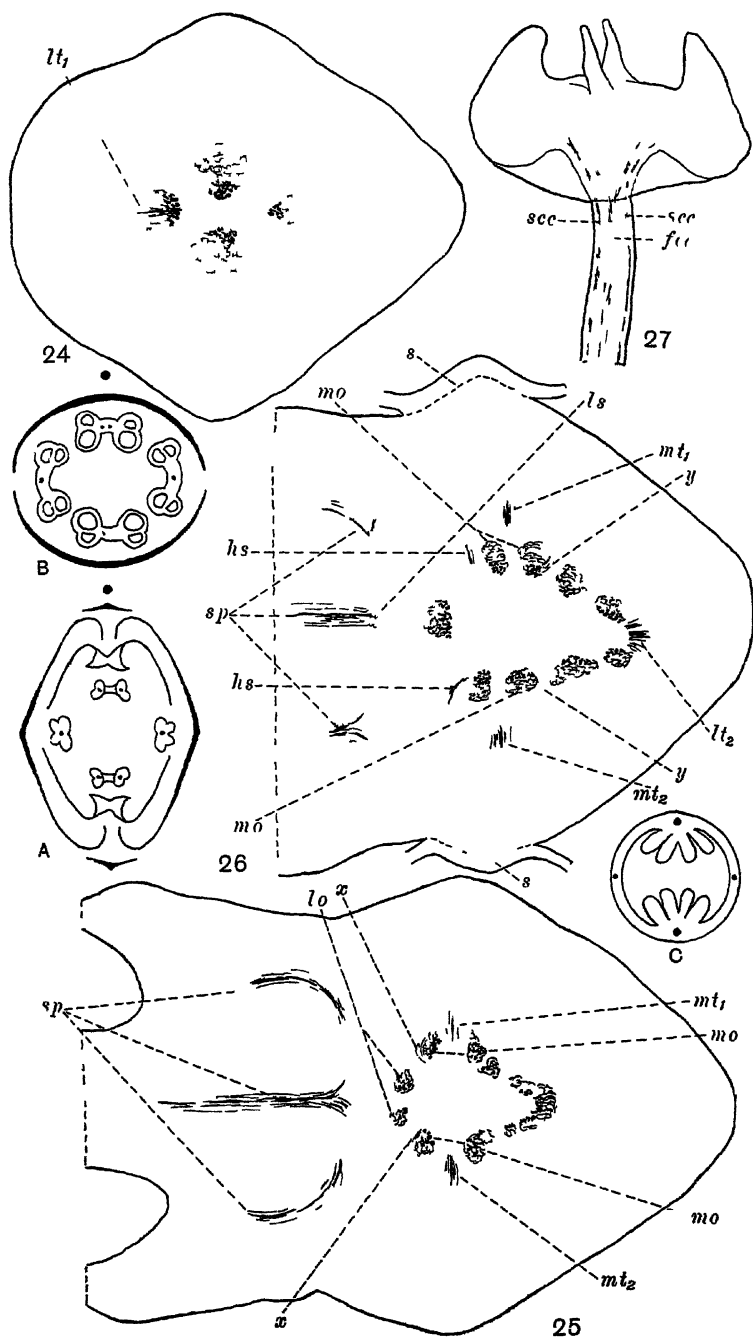
pointed out, the regular alternation characteristic of cyclic flowers occurs between successive whorls of the *foliar members* when their vascular bundles issue separately, but between successive whorls of *trunk cords* when the midrib bundles for superposed whorls issue conjoined together. This arrangement actually occurs in *Pteridophyllum racemosum* Sieb. and Zucc., one of the two genera in the other section (Hypecoideae) of the family, in which the bundles for the stamens arise independently from the central cylinder. Here the four whole stamens, which spring directly from the torus, arise and stand in the diagonal planes¹. In *Hypocoum* there are also four stamens, two lateral, two median, each with four pollen sacs. The two pairs are similar in form and nearly identical in size. The filaments are free though the anthers are syngenesious. In the material which I examined of *H. procumbens* L. and *H. grandiflorum* Benth. each stamen received one vascular strand detached from a petal midrib. The whole stamen complement evidently corresponds to two dimerous whorls in the orthogonal planes, thus continuing the regular alternation exhibited by the perianth whorls. In an account primarily concerned with the contrivances for pollination, Hildebrand states that the connectives and filaments of the median pair of stamens in the former species sometimes show two vascular strands, whereas the lateral stamens have always a single strand (see Diagram B, p. 209): also that the two halves of the anthers of the median pair sometimes diverge at the apex (14). These statements are later repeated by Eichler (12), (see Diagram A, p. 209), Hildebrand having called his (Eichler's) attention to the fact (*loc. cit.*, p. 428) that no mention of these variations had been made by him in his earlier paper dealing with the Fumariaceae (11). Neither of these variations appeared in the material which I examined. Whether Hildebrand's observation indicates a feature of common occurrence in otherwise normal flowers is uncertain. But in any case there can be little doubt that one sees here in an incomplete stage the halving process which in the Fumarioideae has become complete, and that the four filament bearing half anthers in this latter section result from the congenital splitting of two median members. This interpretation is supported by the evidence furnished by the vascular system in *Corydalis lutea* DC. Immediately below the flower the pedicel of this species shows in cross-section four large orthogonal vascular bundles separated by comparatively wide medullary rays (Fig. 24). At the flower base the middle sector of each

¹ Not, as incorrectly represented in Curtis's *Botanical Magazine* (144, Tab 8743, Fig 2, 1918), in the orthogonal planes.

of these four bundles turns outwards as a trunk cord, the two new median cords thus formed dissociating later into the midribs of the sepals and inner petals, the two new lateral cords furnishing the midribs of the outer petals and superposed (lateral) stamens. It is to be noted that the presence of a large nectary on one side only has had the effect of causing the premature departure of the lateral trunk cord on the side on which the nectary and spurred petal occur. So considerable is this effect that the trunk cord for this petal and superposed stamen turns out from the central cylinder even before the median cords for the sepals and superposed inner petals. That this "deformation" of the lateral petal whorl is due to this cause is shown by the fact that the cord for the opposite unspurred petal arises in due sequence, i.e. after, and above, those serving the sepals (see Figs. 25, 26). In the meantime it has become possible to observe the genesis of the strands which will supply the succeeding whorl of four half stamens, for the elements at the point of origin of these strands

Figs. 24-27 and Diagrams A, B, C. *Fumarioideae*. 24-27. *Corydalis lutea* DC. 24-26. From transverse sections of the flower taken at successively higher levels. 24. Flower base showing the four orthogonal cords which pass up from the pedicel. The middle sector of the left orthogonal cord is about to turn outwards to furnish the trunk cord for the lateral spurred petal and the superposed stamen. 25. The same at the level at which the middle sector of the two median orthogonal cords has turned out from the central cylinder to furnish the trunk cord for each sepal and superposed petal (The trunk cord for the right unspurred petal and superposed stamen has not yet been organised) At x elements derived from the inner face of vascular bundles representing residual portions of the two original median orthogonal cords are seen curving round these portions in order to issue from the central cylinder and pass into the two half stamens on the left. 26. The same at the level at which the cord for the right unspurred lateral petal and superposed stamen leaves the central cylinder. At y the elements for the two right half stamens derived from that residual portion of the original median orthogonal cords lying to the right of the median line are curving round these portions in order to leave the central cylinder for the two half stamens on the right. 27. Upper portion of the style filament with the two large lateral and two small median horn-like projections of the stigma rendered transparent and viewed from the front. To right and left the midrib bundles of the two lateral valve carpels; in the centre the midrib of the front consolidated carpel. Diagrams: A, B. *Hypecoum procumbens* L. A. calyx, corolla and androecium (after Eichler). B. Inner petals and androecium (after Hildebrand). C. *Dicentra formosa* Walp. Ovary (constructed after Payer).

fc, fertile carpel cord; *hs*, half stamen bundles; *lo*, residual portions of original lateral orthogonal cords; *ls*, lateral stamen bundle; *lt*, lateral trunk cord for spurred petal and superposed stamen; *lt*₁, lateral trunk cord for unspurred petal and superposed stamen; *mo*, residual portions of original median orthogonal cords; *mt*₁, *mt*₂, residual portions of original median orthogonal cords; *s*, sepal; *scc*, sterile carpel cord; *sp*, spurred petal bundles; x and y as above.



Figs 24-27

have already begun to take a horizontal course. Unlike the preceding set of four orthogonal cords, they are not formed of *sectors* of the residual masses but arise from those elements of the remaining portions of the two original median vascular masses *which about on the pith* and, curving round the side of these masses, make their way out of the cylinder (see x in Fig. 25 and y in Fig. 26). Each median pair of these four strands was observed to arise from the same *original* vascular mass, but as the two strands of each such pair are derived from elements on the inner face of this mass and not from a complete sector they must make their way, as they do, round one side or other of the residual portions of this mass. The result is two pairs of small strands situated on intermediate radii in place of a single bundle in the mid-line back and front.

Now A. Arber's argument derived from the vascular anatomy is as follows. The four half stamens into which the four strands in question pass cannot be regarded as true half stamens because at no stage can one point to the existence of two isolated median bundles which by division give rise in each case to a pair of strands. Further, the fact that in *Corydalis nobilis* and in *C. bulbosa* these four strands were observed to arise from all the four orthogonal bundles to be seen just below the level at which the perianth bundles turn outwards, one strand from each bundle, is held to be conclusive proof that the four half stamens which they serve cannot correspond to the separate halves of two median members. Figures are given for both species of the stage before any strands have turned out from the central cylinder, and of the flower at a level at which the bundles for all the different floral members are already formed and distinct (⁽⁶⁾, Fig. 1, B₁, B₂ and Fig. 3, A₆, A₇), but the critical intervening stages during which the allocation of the different elements to their proper bundles, in other words, the process of organisation, takes place are not shown. It appears, however, from the description that the precise manner in which the process is carried out is not identical in these species and in *C. lutea*. It is further to be observed that although the half stamens and the carpels are symmetrically arranged, the vascular strands which serve these whorls show no symmetrical relation to the median and lateral planes in either *C. nobilis* or *C. bulbosa* *until this reconstitution is complete*. For these reasons it does not appear to me that in these cases any vital significance can be attached to the precise point at which the strands for the half stamens become detached from the products of the breaking up of the original pedicel bundles.

A. Arber's other objection to my interpretation is that at no point

do we find an isolated bundle dividing into twin strands to furnish those for one median pair of half stamens. This again appears to me a fact of no significance in view of the asymmetrical manner in which the elements of the central cylinder become allocated to the outgoing strands. In the flowers which I examined of *C. lutea* a strand destined for the gynoecium remained conjoined with the vascular elements for each half stamen until the moment when these elements turned horizontally outwards. When the relative positions are as shown in Fig. 25, one can understand why the recombining of the elements which serve one pair of half stamens into a single bundle, after they have become dissociated from the strand serving the gynoecium, fails to take place, owing to the conditions of space, and, perhaps, of time.

But apart from the above facts of vascular anatomy, which plainly are in no wise incompatible with the view which I have expressed above, we have other morphological evidence which affords complete confirmation of this latter view. It is noteworthy, for example, that in *Corydalis* the short free region of the filaments of the half stamens is only about half as wide as the filament of the whole stamen; this is also the case in *Dicentra canadensis* Walp. These members evidently have only half filaments as well as half anthers. Then there is the interesting case of virescence in *Dicentra spectabilis* recorded by Wydler (²⁴, p. 290), in which the lateral whole stamens appeared as whole leaf blades, and the half stamens as halved, unequal-sided leaf blades which, as he states, obviously corresponded with the halves of leaves divided down the middle line. More convincing proof that the half stamens are *in reality* half stamens could hardly be forthcoming. Furthermore, in the one other family in which the androecium members are regarded unquestionably as half stamens, viz. the Malvaceae, we have entire confirmation of this view. Here, as in the Fumarioideae, the vascular elements for each of the antepetalous paired groups never constitute an individual bundle, but both in the central cylinder and in the later stages of development are separated by the bulky group of elements for the petal¹.

One further point deserves passing mention, since failure to appreciate its significance *might* lead to its being advanced in support of an incorrect inference. According to Delile (⁹) the filaments of one pair of stamens in *Hypecoum dimidiatum* are winged at the base, as in other species, on *both* sides, each almost completing a circle, while in the other pair the filaments only extend round a half circle, being

¹ The evidence on this point is more fully discussed in my account of the Malvaceae (not yet published).

winged on *one side only*. Now this observation might be cited as an argument in favour of the supposition that, if the full circle pair were the median pair, this pair did not represent two *originally* whole median stamens but two complete stamens formed by the convergence and fusion in the median plane of two diagonal whole members with filaments of the same one-winged form as in the other pair, hence the cause of the presence of two wings in the alternate pair. But it must be noted that Delile does not mention which pair has the whole circle form and which the half circle. Fedde (13), who comments on this observation, remarks that he was not easily able to establish to which whorl the half-circle filaments belonged, though it appeared to him that it was to the outer (= lateral) pair. Now the cause of this uncertainty becomes clear when it is realised that in consequence of the union of the anthers, all four filaments do not lie flat in a pressed specimen. If each of the two filaments of one pair extend laterally to almost a full circle they will appear bilaterally symmetrical whether the flower happens to be compressed in the median or the lateral plane, but the pair whose members each extend laterally only to a half circle will lie flat in the one case and be folded in half lengthwise in the other. In the latter case, although they may in reality be two-winged, they will appear to be one-winged. As in any dried specimen the flowers will presumably suffer compression in different planes, it is unlikely that it will always be the same stamen pair which becomes folded. Herein no doubt lies the cause of Fedde's uncertainty, and of the absence of precise statement in the original account. In the dried material which I examined of various other species, all four filaments were two-winged, although one pair always, on account of folding, had the appearance of being one-winged.

Finally, with regard to A. Arber's statement that she finds it "impossible to follow Čelakovský (8) in regarding the lateral stamens as belonging to an outer whorl, and the monothecal stamens to an inner whorl" (6), p. 349), it is to be said that there is no question but that the lateral stamens do, in fact, constitute an outer whorl in the Fumarioideae, for the trunk cords, consisting of the elements for each lateral petal and superposed stamen invariably leave the central cylinder considerably earlier than the strands for the two half stamens on the corresponding side. The relative position (in respect to whorls) of the lateral and half stamens is determined by the time at which the vascular components of each set leave the central cylinder. This relation is not altered by the fact that the lateral stamen bundles are carried out conjoined with the lateral petal

bundles and do not become disjoined until later (see also the evidence cited above in the discussion on the Cruciferae relating to the behaviour of such trunk cords (p. 184)). We should indeed be faced with some surprising conclusions if this writer's contention were well founded that, as the strands for the half stamens are "individualised" in the central cylinder before those for the lateral stamens become dissociated from the lateral petal bundles already lying well outside the central ring, it is not possible to regard the lateral stamens as constituting an *outer* whorl. Argument on these lines would lead to the paradox, for example, that in those cases in which all the stamen bundles are carried out from the central cylinder conjoined with the perianth midrib bundles, as occurs in many families, these whorls, despite the evidence of our eyes, could not be held to constitute earlier whorls standing *outside* the gynoecium because perianth and stamen bundles do not become dissociated until long after delimitation of the vascular system of the gynoecium is complete. It is unnecessary to cite further instances in order to expose the fallacy of such reasoning.

(3) *The gynoecium.* Little needs to be added regarding carpel number in the Fumarioideae to what has already been stated concerning the typical Crucifer, for the gynoecium is obviously constructed on the same plan in the two families, and in both the whole of the evidence is in accord with a tetramerous interpretation. There are, however, two features in the Fumarioideae giving further support to this view which call for mention. In many types the style filament develops *four* stigma structures. This is conspicuously the case in *Corydalis lutea*, in which the style terminates in an expanded pollen-collecting collar beyond which rise four horn-like projections, two, small in the median, and two, large in the lateral, plane. All four carpel midribs come to an end at the base of the two larger projections (hence no doubt their larger size), the two lateral (sterile) carpel bundles continuing to the end in their original direction, those of the median (fertile) carpels bifurcating at the style apex, the two halves of each bundle diverging and coming to lie alongside the lateral carpel midribs (see Fig. 27). Were only two carpels present the two median prolongations would be entirely unexplained. Further, in certain species of *Dicentra* (*D. formosa* Walp.) *four* rows of ovules are developed on each placenta (Diagram C, p. 209). Were the gynoecium composed of two valve carpels only, analogy with other types would lead us to expect not more than one row of ovules on each margin of each carpel, i.e. two rows on each placenta. A. Arber's support of a

dimerous interpretation is based entirely on certain facts of vascular anatomy, and her account of these facts shows the same misconceptions as are to be found in her description of the Crucifer gynoeceum. She writes (⁽⁶⁾, pp. 343, 344): "Current morphological notions would lead one to expect to find, after the departure of the stamen traces, a residual vascular cylinder, from which the carpellary traces would be derived. But no such cylinder exists." And later: "According to those canons of orthodox morphology which depend on the postulate that stem and leaf are fundamentally distinct entities, a foliar strand cannot arise except from an axial strand, and therefore a carpellary strand cannot be derived from a stamen strand. But if we put aside any such preconceptions about what *should* happen, and simply look at the facts as they are, we cannot fail to recognise (in Fig. 10, B₃ *a* and Fig. 11, B₃ *b* and B₄ *a*) that the process of detachment of a gynoeceum strand from a stamen strand is actually occurring. In order to reconcile the facts with the canons of 'stem and leaf' morphology we should be obliged to describe the median stamen strand in B₄ *a* as having originated from the small strand adjacent to it labelled 'embryonic strand for gynoeceum.'" (Here the reader will note the complete inversion of the order of evolution.) "Fig. B₃ *a* must then be taken to represent the level at which the stamen bundle is 'given off' from this strand. But as the strand in question is in fact merely a meristematic outgrowth from the phloem of the stamen bundle, to regard it as the parent strand of the stamen bundle is clearly absurd. Adhesion to the stem and leaf then obviously leads to an untenable position." As one reads the above lines one has the same feeling as when one views an object with which one is familiar through a distorting mirror. I know of no "current morphological notions" which require the presence, after the departure of the stamen traces, of "a residual vascular cylinder from which the carpellary traces would be derived." Such a cylinder is often, though neither necessarily, nor invariably, present. Nor should I, previously, have conceived that any observer would so reverse the natural order of events as to describe a gynoeceum strand as "giving off" a stamen strand. The whole argument, based on misconceptions, sets out to destroy a chimaera. Nowhere, in her accounts, does this writer appear to appreciate the conception of the trunk cord. In a very great number of Dicotyledon families it is characteristic for the vascular elements for the members of one or another whorl to leave the central cylinder conjoined with those for the members of an outer whorl. I have already instanced a variety of different cases in dealing

with this same question in the earlier discussion of the Cruciferae (see above, p. 183). The degree of differentiation of the issuing strand will naturally depend upon the precise stage of development of the bud or flower examined. It is often the case that when trunk cords undergo dissociation the components for the outer and inner whorls are unequally differentiated. For example, when the trunk cord supplying a perianth member and a superposed stamen is dissociated into its components, the strand for the outer perianth member may be well differentiated, while that portion detached for the stamen may still be undifferentiated. The same relation, as is only to be expected, may hold when the components are those destined, as in the case in question, for a stamen and a superposed carpel. The contradiction which A. Arber sees in this conception of trunk cords lies, not in the conception, which I have presented in some detail, but in the completely reversed form which it takes on in her hands.

The detailed study of the vascular anatomy brings home to us our entire ignorance of the processes of organisation. In a genus with numerous species we may find the same floral ground plan in all. The number of whorls and the number and position of the members of the different whorls may be the same in all the types. Yet the aggregation and allocation of the vascular elements into the midrib bundles leaving the central ring may differ from species to species. We cannot as a rule predict which elements will be those to turn out horizontally, nor can we foretell how those that are left behind in the centre will become rearranged after the exit of the bundles for each whorl. We know equally little about the causes which determine the pattern of the secondary veins and their further ramifications. This pattern varies from species to species and to some extent from individual to individual, though the basic ground plan may be identical. It is for this reason that I doubt whether in the present state of our knowledge any deductions can safely be drawn from certain of A. Arber's observations on the point of connection of the outgoing midrib bundles with the strands of the central ring, or from the later anastomoses between one main bundle and another. Variations of this nature may, it seems to me, have as little significance in regard to the floral ground plan as variations in the finer ramifications of the circulatory system of an animal have in relation to the general scheme of the main blood-vessels.

SUMMARY OF COMMENTS ON A. ARBER'S CRITICISMS OF THE THEORIES OF THE LEAF-SKIN AND OF CARPEL POLYMORPHISM IN ITS APPLICATION TO THE CRUCIFERAE AND FUMARIOIDEAE, AND CONCLUDING REMARKS

The present account sets out to show:

(1) That the above-mentioned writer's argument that the conception of the Leaf-skin should be abandoned and replaced by that of the antithetic relation of root and shoot is illogical, as is also her contention that these two morphological categories should alone be recognised.

(2) That in the instances cited by her as proving that the axis can be terminated by a foliar member the facts do not bear out her argument.

(3) That her account of the mode of origin of the venation system of the Crucifer calyx misinterprets the appearances observed which are described in an order the reverse of that of the evolutionary development.

(4) That her account of the relation in the Cruciferae of the median and lateral pairs of sepals in a few selected species having large lateral nectaries gives a one-sided picture of the range of these relations within the family. It may be said that since in types with well developed median as well as lateral glands the two pairs of sepals are approximately equal and the four midrib bundles take their rise at the same level, there appears to be ample reason for regarding the fundamental ground plan of the calyx as consisting of a single tetramerous whorl, which undergoes "deformation" in those types with an isobilateral disposition of the nectaries. Incidentally it is recalled that, as set forth in my earlier account, the inner staminal whorl should also be expressed as A_4 (not A_2^2), and that, in short, the whole of the evidence supports the view that the fundamental ground plan in the Cruciferae is represented by $K_4C_4A_4 + 4G_4$ which in most types is fully realised except for the two median outer stamens.

(5) That the suggestion that in the Fumarioideae the two outer and two inner petals and the two lateral stamens should be considered from the point of view of whorl succession as together constituting merely a single complex orthogonal whorl, and that the succeeding four half stamens should be regarded as an alternate diagonal whorl of whole stamens, which have lost two out of the normal four pollen sacs (and, it must be added, half their filaments), and that these are followed by a dimerous gynoeceium is against the weight of evidence *in every particular*. That it is altogether at

variance with the clearly observable spatial relations of the members of the perianth and androecium, that it ignores certain features of the gynoecium which are incompatible with it, and that it is opposed to the inferences to be drawn from the structure of the flower in the forms most nearly allied. That, on the other hand, the evidence from all sources points to the rational ground plan $K_2C_2 + 2A_2 + 2$ (split) G_4 (of two pairs).

(6) That various arguments advanced against the theory of Carpel Polymorphism are based on misconceptions of the theory with resulting misrepresentation of the implications involved.

(7) That these arguments are supported by special pleading, appeal to authority and unwarranted assertions.

(8) That the view adopted by the above-mentioned writer that the syncarpous gynoecium, unlike the apocarpous gynoecium, should not be regarded as composed of carpels, and hence that the number of carpels cannot be stated, is as illogical as it would be to maintain that the gamopetalous corolla, unlike the polypetalous corolla, should not be regarded as being composed of petals.

(9) That her view that, nevertheless, for the purposes of description the carpel terminology should be retained and that the number of carpels should be considered to be two is built upon arguments that offer no acceptable solution of certain familiar difficulties in the way of a dimerous interpretation, and upon assumptions for which there is no real evidence.

(10) That the whole vascular anatomy of the gynoecium is treated from the same erroneous standpoint as that adopted in the case of the calyx of the Cruciferae, the appearances observed being related to events which are treated as happening in an order which reverses the order of development. That, furthermore, in the Cruciferae as in the Fumarioideae, other appearances at variance with a dimerous interpretation are ignored.

(11) That the conception that the commissural position of the stigma in the Cruciferae results from "promotion" of the ovule-bearing marginal veins to a "new status" takes no account of the many other cases of commissural stigmas in which styles or stigmas occur over *both* the midribs *and* the supposed "promoted" margins of the carpels; or of the striking case of *Eschscholzia*, in which the styles and stigmas range in number from 2 to 20, and therefore may be many times more numerous than the sum total of midribs and ovule-bearing marginal veins as recognised on the dimerous interpretation of the gynoecium.

As a general comment on the position taken up by the above-mentioned writer, it may be added that her arguments appear to overlook the fact that the morphological generalisations which she attacks are not abstract propositions but are the outcome of observation of facts extending over a wide field. That, nevertheless, she does not hesitate to make morphological assumptions wholly unsupported by evidence where her conceptions demand it. Further, that although for the purpose of controverting an interpretation at variance with her own she argues that deductions drawn from the evidence afforded by the vascular system, carried to an extreme, lead to a *reductio ad absurdum*, she relies practically entirely on evidence of this nature to support her views. In regard to the particular example cited by this writer of such result, it is pointed out that the supposed absurdity is, nevertheless, a demonstrable fact.

It is made clear that no single fact brought forward by the above writer is at variance with, or controverts, either the theory of the Leaf-skin or that of Carpel Polymorphism. In regard to the latter theory it may, indeed, be claimed that, though it may well not represent the final word on the interpretation of the gynoeceum, it, at least, affords a basis which enables us to piece together the known facts more satisfactorily and consistently than any other suggested explanation.

The present account further deals with a certain amount of new evidence and the inferences to be deduced therefrom, regarding:

(a) The origin of the sepal marginal veins in various dichlamydeous families (Cruciferae, Caryophyllaceae, Primulaceae, Oxalidaceae, Geraniaceae), and of the perianth marginal veins in some monochlamydeous types (Sterculiaceae, Phytolaccaceae).

(b) The variable relation between the bract scheme and the orientation of the outermost floral whorl in certain tetramerous types according to the degree of development of the bract complement (illustrated from the Cruciferae, Ericaceae and Phytolaccaceae).

(c) The widespread occurrence of the formation of the midribs of the successive floral whorls, not from bundles which issue as such from the central cylinder, but from trunk cords which later become dissociated into their components (illustrated from the Cruciferae, Caryophyllaceae, Primulaceae, Leguminosae, Piperaceae).

For the drawing of the figures here reproduced I am greatly indebted to Miss D. F. M. Pertz.

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REVIEWS

Manx Algae, an algal survey of the south end of the Isle of Man. By M. KNIGHT and M. W. PARKE. 147 pages, 2 maps and 19 plates. L.M.B.C. Memoir No. XXX. The University Press, Liverpool. 10s. 6d.

Though of modest dimensions, this volume contains an astonishing amount of valuable matter. It is primarily a "local flora" for the marine algae of the south coast of the Isle of Man, but it also gives as full information as possible about the biology of the Manx algae, particularly in relation to the changing seasons. The work is based on the observations made by Miss Knight during the very numerous periods when she has been working at the Port Erin Marine Biological Station, and on the work continuously carried out by Miss Parke who has been for some time past resident algologist at the Station.

The algal species are roughly grouped into perennials, annuals, and "pseudo-perennials"; the latter are species which for the most part live for only a short time, but in which some plants survive the winter as persistent bases. A most interesting seasonal migration of the smaller algae up and down the littoral belt is also described; many species, such as *Rhodomela subfusca* and *Cladophora rupestris* show a downwards (seawards) migration in the winter, a movement tentatively ascribed to the higher temperatures of the lower shore levels in winter: other species such as *Dumontia incrassata* and *Scytosiphon lomentarius*, move, however, in the contrary direction, and in this case the algal movement up the shore in winter is provisionally attributed to the effect of diminishing light intensities at this season. These conclusions on the nature of seasonal migrations of the littoral algae are based on frequent observation of the flora as a whole, and one hopes they may ultimately be supplemented by observations of the growth, reproduction and death of individual plants.

For the assistance of students there is a key to all the Manx genera of algae. This key uses few special terms, and contrives to avoid making use, at least in its early stages, of characters concerned with the reproductive organs. This is a feature reflecting the extensive field observation and teaching experience of the authors, for reproductive organs are only too frequently absent and especially so from specimens collected by students. One of the reviewers tested the key with a dozen slides of small algae and was able to run each of them down to its genus in two or three minutes (all of them rightly), a very unusual experience with a key to a large group. There is no key to species, but for each there is a reference to the best figure and description available; where the existing figures are inadequate new illustrations and notes are given.

In the following section are given critical notes about the life cycles and morphology of twenty of the Manx species, and though they all represent a great deal of very remunerative observation, space does not allow reference to more than two of them. The first is a preliminary account of the life cycle of *Asperococcus* in the Isle of Man. It is based on work by Miss Blackler and shows that *A. fistulosus* is a short-lived alga persisting through the year by a succession of individuals lasting only a few weeks each, and showing a drift in morphology through the seasons. All the typical *Asperococcus* forms are diploid, reproducing by zooids from plurilocular sporangia in winter and from unilocular sporangia in summer. The zooids are respectively diploid and haploid, the latter sometimes conjugating and sometimes not. From the zooids arise small diploid or haploid ectocarpoid filaments which appear to correspond to the "pléthysmothalles" of Sauvageau, and which themselves give rise to zooids (in plurilocular sporangia), which either reproduce the ectocarpoid form or give rise again to *Asperococcus*.

plantules. The second note shows that *Mesogloia* now comes into line with *Laminaria* in having a macroscopic asexual phase (diploid with unilocular sporangia) alternating with a microscopic gametophyte phase (haploid with plurilocular sporangia); the former is restricted to the summer and the latter to the winter.

It appears to the reviewers that the book will be of value along at least three main lines. Students will be greatly aided in the difficult task of naming Manx algae, and if they are at all capable of being enthused many will try to supplement the lists of seaweeds and the knowledge of their reproduction in its complex relations to seasonal conditions. Algologists in other districts will find the information on life histories and on the seasonal biology valuable for comparison, and all ecologists will be grateful for the clear indication of openings to the difficult problems underlying plant distribution in the inter-tidal belt. Finally, the book will be a standard for workers on the Manx algae for a good many years to come, and its precision and exhaustiveness should make it easily possible to determine at any future time what gradual changes have occurred and are occurring in the algal flora of this region, and to a lesser extent over wider ranges of our shores.

T. M. H.

H. G.

A Handbook of the British Seaweeds. By LILY NEWTON, Ph.D.,
F.L.S. Brit. Mus. Pub. 15s.

At least 60 years have elapsed since the publication of any detailed systematic list of marine algae for the British coasts. During this period, though considerable progress has been made in our knowledge of the organisation and life histories of members of this group, and though species new to the British flora have been added to the records, yet general systematic study has been hampered by lack of an algal flora.

In 1902 E. A. L. Batters compiled a comprehensive list of all species at that time recorded, together with their localities, and published it as a *Catalogue of Marine Algae*, without, however, attempting any description of the species recorded. The catalogue, therefore, though of great value to the algologist who was already familiar with the algae, or who had access to the requisite literature, left the general student unprovided for. The accumulating interest in algological studies during the past few years has emphasised the increasing need for a handbook of marine algae. This need has now been met by Prof. Newton and from the outset her book is assured of a welcome.

The book comprises 478 pages, of which the greater part is devoted to a systematic treatment of about 750 species of algae occurring round the coasts of the British Isles. An introduction, which might with advantage have been made more comprehensive, an abbreviated glossary and an index, together with 270 figures in the text complete the book.

The nomenclature and scheme of classification adopted is largely that followed by Batters in 1902, though Prof. Newton has not scrupled to depart from Batters' scheme in many places, especially in the naming and grouping of the Rhodophyceae, where the recent proposals of Kylin have been accepted without criticism or comment. Where other changes of nomenclature have been made it would have been an advantage to have had a brief statement of the reasons for the change.

Information relating to the derivation of names is a pleasing feature, and the introduction of artificial keys to the identification of genera and species will appeal very much to all students. The building of these keys must have been a difficult task and must have involved a great amount of labour.

In view of the discordance in the descriptions of the species of marine algae and the resulting confusion of nomenclature, especially where families with a large number of species are concerned, it would be too much to expect Prof.

Newton to have produced the perfect algal handbook. There are undoubtedly certain groups with which she has obviously had difficulty and which will probably serve as the subjects of individual treatment in the future. Incidentally, however, there are certain errors which might perhaps have been avoided. For example, the statement that "it takes some twenty or more tons of wet weed to yield one ton of ash" is surely an incorrect estimate of the proportions! The nomenclature is not consistent; sometimes a plant is referred to under the older name as it appears in Batters' list and sometimes under the new name suggested by a later author, for example: Figs. 201 *a* and 205 *a* refer to identical plants, yet one is referred to as *Nilophyllum laceratum* and the other as *Cryptopleura ramosum*.

Prof. Newton herself makes no specific claim to have included all the British species of marine algae (though the preface does), but her treatment of recent records is inadequate. More than 40 British plants—some of them actually new species—recorded by Batters, Cotton and Lyle in various publications have been omitted.

In passing it may be observed that if a glossary were considered to be an essential part of the book it might have been made a little more comprehensive. At one point the discrimination between two easily confused species rests on the distinction in meaning between "lubricous" and "gelatinous," but the former adjective is not defined in the glossary.

The illustrations, for all of which Prof. Newton is not personally responsible, vary in value and some leave much to be desired. Doubtless the limitations of economical publication have imposed restrictions on the type of illustration available, but many of the figures fail to convey to the student any true picture of the plant as it appears in the field. Without the name printed below it few would recognise the figure of *Codium tomentosum*, and the illustration of *Pelvetia canaliculata* bears little resemblance to the familiar plant of high-water mark. Many of the figures illustrating microscopic characters are unconvincing, and some fail to convey any information whatever.

Despite these criticisms, Prof. Newton has undoubtedly conferred a great benefit on all students of botany in thus filling a gap that has been vacant so long—a gap which has proved such a stumbling block to the study of marine algae. The attempt to produce the handbook was a task of great magnitude and Prof. Newton is to be congratulated on the courage with which, single handed, she attacked the systematic treatment of the marine algae, a group in which almost every family demanded complete reinvestigation *in the field* before the discrepant statements in the literature could be tested and corrected. No less an equipment than a lifetime's intimate knowledge of the plants, derived from observation of living material in many fields, could have made possible a completely successful treatment of this difficult group of plants.

Prof. Newton's book will be of great practical value. It will admirably serve the needs of general students of botany and will also act as a stimulus to advanced workers, so that one may confidently predict that the next few years will see a steady stream of detailed monographs dealing with the less well-known groups of marine algae.

M. K.

NOTICES

APPEAL

TO BOTANICAL INSTITUTES AND ALL BOTANISTS FOR
FUNDS TOWARDS THE ERECTION OF A BUST OF

DR JOHN BRIQUET

ON October 26th, 1931, Dr John Briquet, Director of the Botanic Garden and "Conservatoire botanique" in Geneva, died at the age of 61, after a brief illness. This painful loss must awaken sincere regret throughout the botanical world and arouse feelings of deepest sympathy for his family and the institutes with which he was so long and honourably connected. In his death botanists have lost a true friend, a lovable, highly accomplished man and a recognised authority; one whose scientific conscientiousness is reflected in all his published works.

All who have attended meetings of the International Botanical Congresses—in 1900, 1905, 1910 and 1926—will realise the great loss botany has suffered by his death. At those meetings he was an outstanding figure in all discussions on Nomenclature, and the rôle he played as recorder, by his tactful, sagacious and conciliatory nature, together with his great knowledge of languages and absolute command of the matter in hand, left an indelible impression on the minds of all. Thus he contributed greatly to the unifying of botanical nomenclature, and his services at the memorable Congress held at Cambridge in 1930 will long be gratefully remembered.

The undersigned, personal friends and colleagues of Briquet, representing the botanical circles of his native land, desire to commemorate his great services to our science by the erection of a *bronze Bust* to be placed in the "Conservatoire botanique" in Geneva, along with those of Vaucher, de Candolle, Boissier, Ascherson, Engler and others who have done so much to enrich the herbarium of the Conservatoire, to which Briquet has contributed in so large a degree. We feel sure it is the desire of all botanists to bear this testimony to the very great services he rendered to that botanical institute, as well as to his invaluable and ever willing help to all who used the herbarium. Together with de Candolle, Delessert, Boissier and Chodat he made Geneva a Mecca to all botanists.

We shall be glad if you will show your appreciation of the life-

work of Briquet by contributing to the above memorial. Subscriptions should be sent to M. le Prof. E. Wilczek, Palais de Rumine, Musée botanique, Lausanne.

Dr H. CHRIST, Riehen near Basle.

Dr B. P. G. HOCHREUTINER, Director of the Botanic Conservatory, Geneva.

M. OECHSLIN, President of the Swiss Botanic Society, Altdorf.

Prof. Dr E. RUBEL, Central President of the Helvetic Society of Natural Sciences, Zurich.

Prof. Dr H. SCHINZ, Emeritus Professor at Zurich University.

Prof. Dr C. SCHRÖTER, Emeritus Professor at the Federal School of High Technical Studies, Zurich.

Prof. Dr E. WILCZEK, Conservator of the Botanic Museum, Lausanne.

ALTDORF, BASLE, GENEVA, LAUSANNE AND ZURICH,
March, 1932

SIXTH INTERNATIONAL BOTANICAL CONGRESS

ACCORDING to a decision by the Fifth International Botanical Congress at Cambridge in 1930, the Sixth Congress will be held in Holland in 1935. An Executive Committee has been formed, President of which is Prof. Dr F. A. F. C. WENT (Utrecht), while Prof. Dr J. C. SCHOUTE (Groningen) will act as Vice-President, Dr W. C. DE LEEUW (Bilthoven) as Treasurer and Dr M. J. SIRKS (Wageningen) as Secretary. The Committee has decided that the Sixth Congress will meet at Amsterdam, September 9th-14th, 1935. Scientific Societies are kindly requested to reckon with these dates in planning their own meetings.

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CROSSES BETWEEN *DIGITALIS PURPUREA* AND *DIGITALIS AMBIGUA*

By B. H. BUXTON AND C. D. DARLINGTON

(With Plates VI–VIII and 4 figures in the text)

IN the *Journal of Genetics*, vol. 19, p. 269, 1928, B. H. Buxton and the late W. C. F. Newton published a preliminary account of certain crosses between *Digitalis purpurea* and *D. ambigua*, and the summary of this is here given as an introduction to the present article.

"A number of hybrids between *Digitalis purpurea* ♀ and *D. ambigua* ♂ were obtained. Two plants among the hybrids showed a low degree of fertility¹. From the others no seeds were obtained.

"The F_2 generation differed from the F_1 principally in size, but there was no segregation of the parental characters. About 75 per cent. were highly fertile.

"The chromosome numbers of the parent species and of the F_1 hybrid are 28 (n) and 56 ($2n$) in each case. In the F_2 hybrid (artificial pollination) the numbers are 56–112, while all plants obtained from open pollination of the F_1 are triploid (84)² and sterile³.

"The viable spores of the F_1 result from the failure of the reduction division and the formation of restitution nuclei.

"*Digitalis purpurea* × *ambigua* is an additional example of a constant intermediate hybrid resulting from chromosome doubling."

In the present paper the senior author is responsible for the genetical observations, while the junior author has made the cytological studies at the John Innes Horticultural Institution.

INTRODUCTION

The cross between *Digitalis purpurea* and *D. ambigua* has been made several times before. A detailed description of the hybrid and

¹ From one of which the fertile tetraploid was raised, from the other only sterile triploids by natural pollination (crossed with *D. purpurea*).

² Or approximately so.

³ They are back-crosses with the parental species growing in the neighbourhood.

its reciprocal (showing important differences attributed to the influence of the cytoplasm) has been given by Neilson-Jones (1912), as will be seen, his *grandiflora* differs in certain respects from our *ambigua*. In this account it was also shown that when the white foxglove (*D. purpurea* var. *alba*) was used as a ♀ parent and the yellow-flowered species as ♂, the hybrid had pink flowers; this we have also found.

We find nine recorded cases of the cross *D. purpurea* × *D. ambigua* in the literature, besides two personal communications. In only one instance is an F_2 mentioned, a single plant (Warren, 1924) which did not reach maturity.

In Neilson-Jones', as in all other recorded cases, the F_1 hybrid has been found to be invariably sterile when crossed *inter se*, although back-crosses with *D. purpurea* have occasionally been successful. We also have raised a few of such back-crosses, but they have always proved sterile.

In 1925, however, as previously recorded, among four plants intercrossed under controlled conditions of pollination, one set a few seeds from which a constant intermediate giant hybrid was raised. We see no objection to regarding this new form as a species, and propose to refer to it as *Digitalis mertonensis*, and specimens under this name have been placed for reference in the Genetical Herbarium at Kew. Apart from its constant habit it is sufficiently diagnosed by its chromosome number of 112, which is at once responsible for its constancy when selfed and its sterility when crossed with either of its grandparents.

The cross *D. purpurea* × *D. ambigua* was repeated in 1929, using several plants, both purple and white, for the female parent. From these an F_1 of about 200 plants exactly resembling the F_1 of the previous cross was reared, of which about 150 have since flowered. Not a single seed could be found among the plants left to pollinate naturally, but out of eight plants isolated and intercrossed (*ca.* 250 flowers) one capsule on one plant was found to contain 12 seeds, which seemed as if they might be viable. Two of these have germinated, and have the appearance of being tetraploids, but are still too small to test further. It is clear from our own and others' experiences that successful doubling takes place with extreme rarity.

The cross *D. ambigua* × *D. purpurea* has never proved successful in our hands, but Neilson-Jones describes his single plant as being nearer to *D. ambigua* than to *D. purpurea*. Other observers also remark on the difficulty of succeeding with this cross.

EXPERIMENTAL

(1) *Controlled pollination of the tetraploids*

From a controlled intercross between two F_2 plants, 81 plants were raised of which 65 flowered in 1930 when two years old; 46 of these set good seed, and 19 were sterile, i.e. the fertility is approximately 70 per cent. A hot dry spell in June seemed to affect the plants very severely, and it is probable that under more favourable conditions a larger proportion would have set good seed.

Among these F_3 seedlings several had flowered already in 1929, and from crosses between four of these plants 82 seedlings have been raised, of which about 30 have flowered, several of the plants having succumbed to a severe frost in the spring of 1931. All these F_4 plants so far as they have flowered resemble their parents, both in leaves and flowers, no segregation being apparent in the F_3 or F_4 any more than in the original F_2 from which they have been derived.

Root tips of the F_3 parents and of the F_4 seedlings have been found to have 112 chromosomes, which form 56 bivalents with some "secondary pairing" at meiosis. In one F_4 plant an additional fragment chromosome was found (usually unpaired).

The tetraploid nature of the direct generation and their fertility seem to be definitely established. Calling the *D. purpurea* chromosome set P , and the *D. ambigua* A , the scheme for the genetics may be taken as (x being the set of 28 chromosomes):

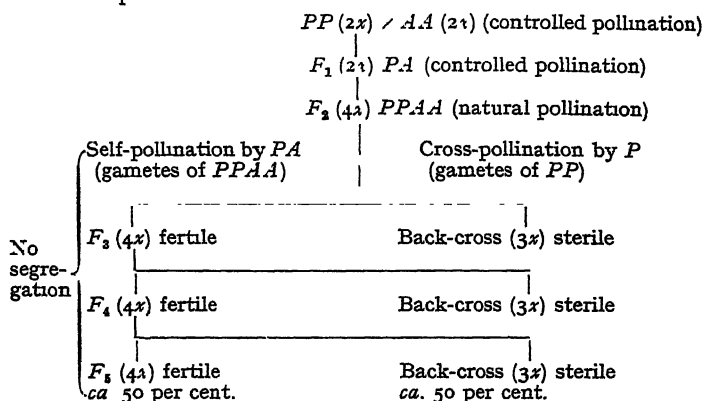
$$\begin{array}{c}
 PP (2x) \times AA (2x) \\
 \downarrow \\
 F_1 (2x) \text{ i.e. } PA \\
 \downarrow \\
 F_2 (4x) \text{ i.e. } PPA A \\
 \downarrow \\
 F_3 (4x) \text{ i.e. } PPA A \\
 \downarrow \\
 F_4 (4x) \text{ i.e. } PPA A
 \end{array}$$

(2) *Natural pollination of the tetraploids*

A large number of plants have been raised from natural pollination of the F_2 and further generations. Without going into details it may be said that from a good-sized batch of second year plants, flowering simultaneously with the ordinary *D. purpurea* in the vicinity, the seed collected gives about 50 per cent. of fertile tetraploids to 50 per cent. of back-crosses with *D. purpurea*. The two classes are sharply distinct, all of the latter being triploid and practically sterile. In this way F_3 , F_4 and F_5 generations have been obtained from the original fertile F_2 , without any control over the pollination.

One instance may be cited. Sixty seedlings in one batch from natural pollination of F_3 gave 35 ($4x$) $PPAA$, and 25 ($3x$) PPA . Naturally the actual numbers vary according to the varying relative numbers of the potential parents.

There seems therefore no reason why the new tetraploid species should not perpetuate itself naturally, since any triploid back-cross would be sterile or very nearly so. It is in this way closely analogous to Karpechenko's *Raphanus-Brassica* tetraploid, which gives sterile triploid back-crosses. The genetical scheme for natural pollination can be drawn up as follows:



It might be thought that this crossing, by spoiling half the seed of *D. mertonensis*, would ruin its chance of survival. But this is not necessarily so. In considering the competition of the old and the new species (for it is only in the presence of one another that this drawback would work) we must remember that it would presumably be equally effective in the opposite direction, i.e. in sterilising *D. purpurca*. Effective reproduction would continue only by self-fertilisation of the true-breeding forms.

(3) Controlled back-crosses

D. mertonensis back-crossed with *ambigua* is very slightly fertile; few seeds are set, and few of these germinate, but 15 of these have been reared from two such crosses; all of them were sterile and triploid, as shown by root-tip examinations, except two, which were approximately diploid ($2n = 58$ and 56 , Plate VII).

Both leaves and flowers of the triploids showed a distinct leaning towards the *ambigua* type, the flowers being a pale pink on a ground of a much deeper yellow than in the F_1 or tetraploids, and their con-

stitution is obviously *PA.A.* Unfortunately the triploid plants all died off before there was an opportunity of examining the buds for the reduction division.

The first diploid plant flowered in 1928. It resembled the F_1 plants and like them was sterile. It set no seeds at all with open pollination. It died in the severe winter of 1928-9. There can be no doubt that it arose by parthenogenesis, i.e. by the development of the unfertilised, relatively haploid egg cell of *D. mertonensis*. The second diploid plant has not yet flowered. Nearly twenty analogous cases have been recorded in the flowering plants (cf. Darlington, 1931 *b*). Their method of origin, the stimulation of development by pollen of another species, is characteristic of parthenogenesis. They are not true haploids. Relative to the presumed basic number in *Digitalis* of 7, they are actually octoploid. But they have a special interest of another kind.

In the genetical history of these plants the orthodox alternation of generations has been set at nought, as far as the nucleus is concerned, for its grandparents omitted reduction and its parent omitted fertilisation.

It has always been assumed that the fertility, constancy and gigantism found in the derivatives of *Primula kewensis*, *Raphanus-Brassica*, *Nicotiana digluta* and the rest followed doubling of the chromosome number as a *consequent*. Logically the objection might always be raised that they were parallel consequents of the same antecedent. In the present case the process is reversed. The chromosome number is halved and the fertility and gigantism disappear. But, since the halving takes place as a result of a known irregularity of development (failure of fertilisation) totally unrelated to the irregularity which led to the opposite effect, the argument of parallelism falls to the ground. The reversal provides a critical test of causality.

In the diploid seedling the chromosomes fail to arrange themselves on the plate. Frequently pairing seems to be entirely absent, but as many as twelve pairs have been seen in the middle of the spindle, the unpaired chromosomes lying evenly distributed over the spindle on either side. An occasional quadrivalent is to be seen. Following the division a restitution nucleus is almost always formed.

In a word the behaviour of the seedling corresponds exactly with that found in the sterile diploid F_1 . It would appear that the sterile diploid has in fact been reconstituted, as nearly as possible, by the failure of fertilisation in the F_2 .

In contrast to the cross *mertonensis* \times *ambigua*, the cross *mertonensis* \times *purpurea* set seed very freely, and a large number of seedlings

have been raised. They show some leaning towards the *purpurea* type, more particularly the flowers, which have little of the *ambigua* strain apparent. Their constitution is evidently *PPA*: all of them are triploid and almost completely sterile. No seeds whatever have so far been set by them under conditions of controlled pollination, but from a large number of capsules on 60 plants under open pollination a few seeds were collected in 1930, and from these 130 seedlings were raised and about 75 plants brought to maturity.

In the triploid back-cross of *D. mertoniensis* with *D. purpurea* (*PPA*), as described in the earlier paper in the case of the natural triploid seedlings, more than 28 bivalents are formed at the first metaphase of meiosis. Autosyndesis therefore occurs to a slight extent within the extra *ambigua* set of the triploid. It might be supposed alternatively that pairing took place between *purpurea* and *ambigua* with autosyndesis in the odd *purpurea* set. But in view of the behaviour of the tetraploid this alternative is not probable.

On account of the large number of unpaired chromosomes which are chiefly scattered over the spindle at metaphase, both the first and the second divisions may prove abortive, giving in the first case the restitution nuclei of Rosenberg (1927). This abortion is less frequent than in the diploid, owing, one would suppose, to the higher frequency of pairing, and second divisions are often observed with approximately half the triploid number, the result of the random segregation of univalents.

A rather remarkable feature in one of the F_2 back-crosses with *D. purpurea* was the appearance of dwarfs. Out of a batch of about 100 plants, six grew very slowly, and remained quite small, but all died off without flowering.

Root tips taken of two of these plants showed in each case 84 chromosomes ($3x$), the same number as in plants of the normal size.

The dwarfing therefore is not due to deficiency in chromosome number, but rather to segregation of parental characters (from the occasional pairing of *P* and *A* chromosomes, as in *Primula kewensis*) producing an ill-assorted complement.

The photograph shows the relative sizes of the normal and dwarf plants in June, 1929, at about 9 months after sowing (Pl. VII, fig. 2).

Four plants also showed an intermediate dwarfish habit, being very much smaller than the normal. The flowers of these were rather small, but essentially similar to those of the normal-sized plants. After flowering they all died out.

To return to the 130 plants raised from open pollination of the

triploids (PP4) a large proportion of these were small dwarfs, all of which have died out. The larger plants have not yet flowered, but the leaves of about 64 of them have been measured for index (see Text-fig. 3). They appear to be a very mixed lot, the indices varying from 30 to 60 (see Table II, and Text-fig. 2), and some of them have very peculiar crinkled leaves, quite unlike any hitherto observed in *Digitalis* plants. It does not seem likely that any of them will be fertile, but if they are it will take some years to work them out.

The somatic chromosomes of six natural seedlings of the triploid were counted and found to be 263, 65, 66, 67, 68 and 77 respectively (the two of these with crinkled leaves had 65 and 67). These numbers show the random distribution of the extra chromosome set of the triploid, expected from behaviour at meiosis; they do not show the sharp elimination of unbalanced types which is characteristic of the progeny of simple triploids. Clearly in a high polyploid disproportion of chromosomes is, as one would expect, a less serious defect than in low polyploids.

(4) *Hybridisation of the new species*

The new species has been crossed with several other species. The results of these crosses will be described later. The hybrid with *D. obscura* (chromosome number presumably 112) has 112 chromosomes.

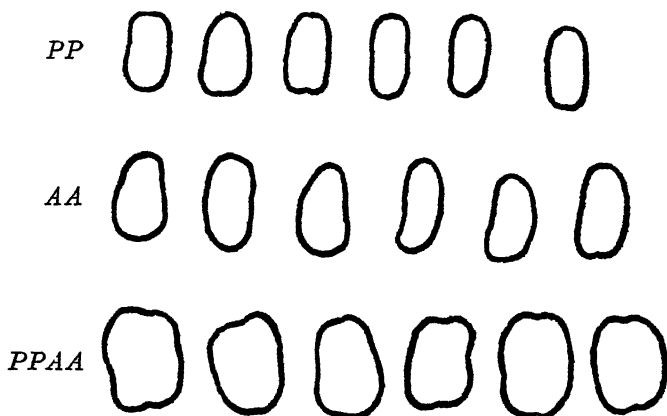
(5) *General observations*

(i) *The seeds*

On screening the seeds through a 1 mm. mesh it is found that all those from *D. purpurea* will pass through; of the *ambigua* seeds about 50 per cent. pass through, and of the tetraploids about 25 per cent., 75 per cent. remaining on the screen. The tetraploid seeds are therefore distinctly larger than either of the others, as is also evident in the drawing (Text-fig. 1). Sowings, however, from the smaller tetraploid seeds yielded a very good crop of seedlings, apparently of growth and vigour equal to those from the larger seeds.

The above tests were made in 1928 when all the foxgloves set very good and plentiful seed. In 1930 an attempt was made to estimate the number of seeds per capsule, but as already mentioned the season was very unfavourable for seeding. The average numbers estimated from 30 capsules in each case, were: *D. purpurea* ca. 900, *D. ambigua* ca. 300, and the tetraploids ca. 400, but in a normal year the number would doubtless be considerably higher, although the relative seed production may approximate to the normal. Since the tetraploid is intermediate between its diploid parents in seed production, as it

would be expected to be in ovule production, its generational fertility may be taken to be as high as theirs. Since the diploid was, as a diploid, absolutely sterile, this is another example of the rule that there is an inverse correlation between the fertility of a diploid and that of the tetraploid to which it may give rise (Darlington, 1928).



Text-fig. 1. Top row, seeds of *D. purpurea*; second row, seeds of *D. ambigua*; bottom row, seeds of *D. mertonensis*. Drawings made from a photograph. $\times 9$.

TABLE I

Fertile diploids, infertile tetraploids	Infertile diploids, fertile tetraploids
1 <i>Oenothera Lamarckiana</i> (Gates, 1915)	1 <i>Primula kewensis</i> (Newton and Pel-lew, 1929)
2 <i>Datura Stramonium</i> (Blakeslee et. al. 1923)	2 <i>Raphanus-Brassica</i> (Karpechenko, 1927)
3 <i>Primula sinensis</i> (cf. Darlington, 1931 a)	3 <i>Nicotiana digluta</i> (Clausen and Good-speed, 1925)
4 <i>Campanula persicifolia</i> (cf. Darlington, 1928)	4 <i>Nicotiana Tabacum</i> \times <i>rustica</i> (Rybin, 1927)
5 <i>Solanum nigrum</i> (Jørgensen, 1928)	5 <i>Nicotiana Tabacum</i> \times <i>sylvestris</i> (Rybin, 1929)
6 <i>Solanum lycopersicum</i> (Jørgensen, 1928)	6 <i>Fragaria bracteata</i> \times <i>Helleri</i> (Ichijima, 1926)
7 <i>Primula malacoides</i> (Philp, unpublished)	7 <i>Rubus rusticanus</i> \times <i>idaeus</i> (Crane and Darlington, 1927)
8 <i>Primula obconica</i> (Philp, unpublished)	8 <i>Solanum nigrum</i> \times <i>S. luteum</i> (Jørgensen, 1928)
	9 <i>Saxifraga potternensis</i> (Marsden-Jones and Turrill, 1930)
	10 <i>Aegilops ovata</i> \times <i>Triticum durum</i> (Tschermak and Bleier, 1926)
	11 <i>Aegilops ovata</i> \times <i>Triticum dicoccoides</i> (ibid.)
	12 <i>Triticum vulgare</i> \times <i>Secale cereale</i> (Levitsky and Benetzkaia, 1929)
	13 <i>Triticum turgidum</i> \times <i>T. villosum</i> (Tschermak, 1930)
	14 <i>Galeopsis pubescens</i> \times <i>G. speciosa</i> (Müntzing, 1930)

Table I gives a list of other cases illustrating the rule, taken from recent experiments.

This rule seems to be equally applicable to the newer cases. In the *Nicotiana rustica* and *Rubus rusticanus* crosses the diploid hybrid was not obtained, but, as an interspecific hybrid, may legitimately be taken as infertile. It may therefore be worth while explaining the apparent cytological basis of the rule. Generational sterility is a symptom of segregation; indeed it may be so defined to distinguish it from parental sterility, which is determined at an earlier stage¹. In interspecific crosses, owing to the breadth of the segregation, sterility is roughly proportional to it. This segregation may be of two kinds: (i) segregation of unlikes²; (ii) lack of segregation of likes (giving unbalanced gametes in polyploids and in structurally hybrid diploids³). It follows therefore that the higher the sterility of a diploid, the greater will be the segregation of unlike chromosomes; and in its tetraploid offspring the greater will be the regularity in the pairing of like chromosomes; the fertility of the polyploid will be increased. On the other hand the higher the fertility of a diploid the more frequent will be the pairing of like chromosomes in fours in the tetraploid and the failure of regular segregation of likes. The fertility of the polyploid will therefore be reduced.

This will be clear from a consideration of Text-fig. 2. In the diploid hybrid even where pairing is perfect dissimilar chromosomes pair and pass to different daughter cells. The three black chromosomes of one species which made a satisfactory complement together will rarely come together. One of them will be replaced by a non-com-

¹ Three kinds of sterility may be distinguished:

(i) *Parental sterility*, determined by morphological or physiological abnormality of the parent plant, e.g. degeneration of sex organs, immaturity, or disease. It is *pre-meiotic*.

(ii) *Relational sterility*, determined by the failure of viable germ cells to meet, e.g. incompatibility of pollen with stigma in self-sterile plants when they carry the same incompatibility factors. It depends on the *relations* of parental zygotes with gametes.

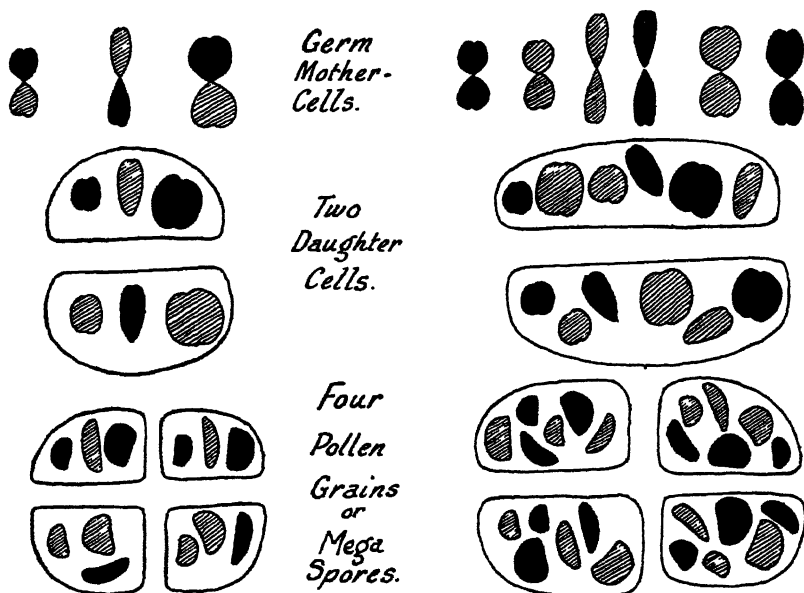
(iii) *Generational sterility*, determined by the segregation of dissimilar chromosomes at meiosis, which will result in the formation of dissimilar and non-viable germ cells. This sterility alone is the essential characteristic of hybrids other than tetraploid hybrids with doubled chromosome numbers. It is *post-meiotic*.

² The terms "unlike" and "like" are here used in the sense that like chromosomes are those which can replace each other in the haploid chromosome content of a given species, and unlike ones cannot.

³ Since the system of segregation in new structural hybrids is different in certain important respects they need not be expected to obey the rule, and they require special consideration. Old-established forms such as *Oenothera Lamarckiana* on the other hand may be treated as normal and therefore agree with the rule.

plementary chromosome of the other species, and the resulting gamete will be non-viable: the hybrid will be *generationally* sterile.

In the tetraploid hybrid on the other hand the dissimilar chromosomes of opposite species stand little chance of pairing with one another in the presence of identical mates. These identical mates pair and pass to opposite poles; the germ cells are therefore uniform and contain a complete balanced set of chromosomes of each species. The new form is fertile.



Text-fig. 2. Diagram illustrating the chromosome behaviour at germ-cell formation in relation to the relative fertility of diploid and tetraploid hybrids with a basic set of three chromosomes (see text).

In the tetraploid which is not hybrid, associations of four chromosomes are formed, which segregate unevenly (into three and one) and the germ cells are therefore of a variety of unbalanced types: fertility is reduced.

The principle therefore depends on (i) competition in zygotene pairing at meiosis in polyploids; (ii) formation of multivalents where more than two like chromosomes are present; and (iii) a qualitative differentiation of the chromosomes of a complement. None of these conditions is universal¹, but since they are all nearly so the principle

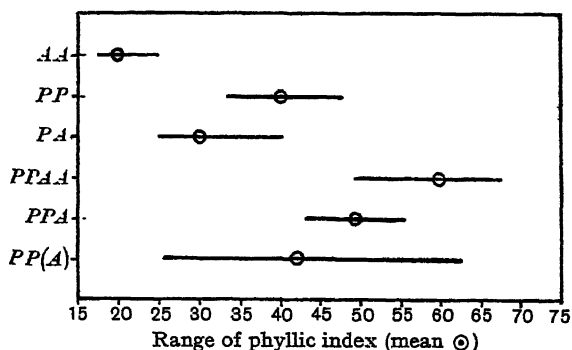
¹ The first condition might not apply to certain structural hybrids; the second condition would not apply with a localisation of chiasmata (Darlington, 1932), and the third condition is less important in high polyploids.

should be widely applicable and particularly in arguing as to the probable constitution of polyploid species, with regard to which in some instances it provides the only evidence so far available of the constitution, whether hybrid or otherwise.

ii) *The rosette leaves*

The leaves of *D. purpurea* are broad with a long petiole, and those of *D. ambigua* narrow and sessile, the lamina springing directly from the base of the leaf. The leaves of the F_1 are intermediate in breadth, usually without a definite petiole, but narrower than those of *D. purpurea* (Pl. VI).

The leaves of *D. mertonensis* are very large, considerably broader in proportion to the length than those of *D. purpurea*, while those of



Text-fig. 3. Diagram to show the range and the mean of the phyllitic index (ratio breadth to length expressed as a percentage) for parent and hybrid *Digitalis* plants of different genetical constitution. *PP(A)* are the natural seedlings of *PPA* and are very variable as expected.

the back-crosses *D. mertonensis* (F_2 or F_3) \times *D. purpurea* are rather narrower in proportion to the length as compared with the tetraploids. On looking over a mixed lot of rosettes arising from a natural pollination of a tetraploid, one can judge from the leaves alone which plants are selfs and which are back-crossed, and such observations are nearly always borne out later on by the character of the inflorescence and by chromosome counts.

On reckoning the phyllitic index

$$\frac{\text{Breadth} \times 100}{\text{Length}} = \text{index},$$

the accompanying figures were obtained. The length is measured from the stem to the tip of the leaf, and the breadth at the widest point about the middle.

TABLE II

	No. of leaves measured	Averages		
		Length in cm.	Breadth in cm.	Index
<i>D. purpurea</i>	50	24.36	9.40	38.70
<i>D. ambigua</i>	50	20.22	4.22	20.90
F_1 (2n)	100	27.94	8.95	32.00
<i>D. mertonensis</i>	100	26.10	15.80	60.50
Triploids, <i>PPA</i>	100	29.21	14.39	49.25
<i>PPA</i> nat. seed (= \wedge <i>PP</i>)	64	22.10	9.30	42.00

Roughly therefore one can consider the indices as:

ambigua, *AA*, 20; F_1 , *PA*, 30; *purpurea*, *PP*, 40; *mertonensis*, *PPAA*, 60; triploids, *PPA*, 50.

The actual lengths and breadths given have not much significance, as the leaves vary so much in size even on the same plant, but the index remains fairly constant irrespective of the size of the leaf.

The measurements were taken in June, 1930, from plants six to seven months old, before there was any evidence of a flowering stem. Some measurements taken in previous years, but less systematically, averaged rather less for the triploids (*ca.* 45) and the tetraploids (*ca.* 55), but for the other three, *purpurea*, *ambigua* and the F_1 , the indices were practically the same as for the figures given above.

As already mentioned in the previous communication, the margins of the leaves differ to a considerable extent. Those of *D. purpurea* are crenate, those of *D. ambigua* finely serrate. The F_1 and tetraploid leaves are intermediate, as is shown in the series of photographs (half natural size) in Pl. VI, figs. 3 and 4. The differences in venation can also be noticed in the photographs.

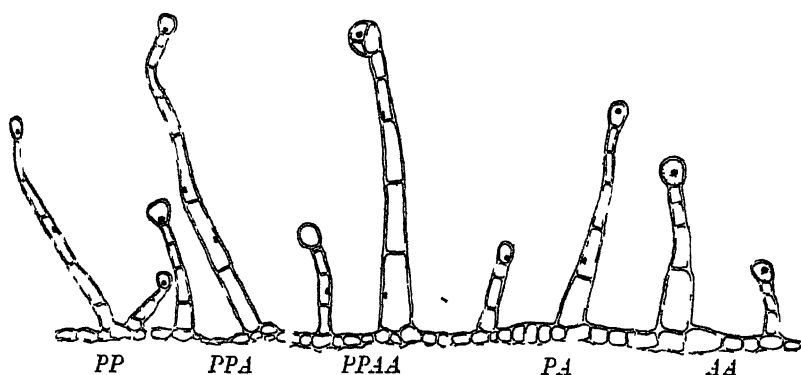
(iii) *The flowers*

Wash drawings of the throat and lower lip of the flowers (Pl. VIII) are sufficient in themselves to explain the differences between them; merely remarking that the F_1 and tetraploids are pink on a yellow ground, instead of purple or pink on a white ground, as in *D. purpurea*. The triploids *PPA* are also pink, but the background is a very pale yellow, whereas the triploids *PAA* are pale pink on a deep yellow ground. In other words the triploids show a distinct leaning towards the parent with which they have been back-crossed. It may also here be remarked that the purple of *D. purpurea* is an anthocyanin, and the yellow of *D. ambigua* a plastid pigment insoluble in water. The pink of the intermediates can therefore be dissolved out in water, leaving the yellow ground intact.



BURTON AND DARLINGTON—CROSSES BETWEEN *DIGITALIS*
PURPUREA AND *DIGITALIS* *AMBIGUA*

The calyx in both parent species is densely pubescent but differs sharply in the type of hairs. For comparison hairs from the margin of the calyx in nearly mature flower-buds have been photographed and drawn. The parental types differ in important respects from those illustrated by Neilson-Jones (cf. his Fig. 17). The apical cells on all the hairs of both of our species appear to be globular, and on those of *D. ambigua* to be well provided with chloroplasts. The lolling (sub-erect) habit of the hairs of *D. purpurea* is characteristic. The hairs of the hybrid types (Pl. VII, fig. 3) show how generalised the differences between the parental species must be, for, as in all other such cases, the properties of the particular organs are determined by the proportions of the parental chromosome sets (cf. Karpechenko, 1927; Rybin, 1927; Crane and Darlington, 1927).



Text-fig. 4. Marginal calyx hairs of the parental species and their hybrid, showing the influence proportional to the number of chromosome sets of each type ($\times 44$).

A floral index has been taken of the flowers, the length being measured from the base to the tip of the lower lip, and the breadth across the middle of the flower. Fifty flowers were measured in each case.

	Averages of 50 flowers			Area factor sq cm.
	Length cm.	Breadth cm.	Index cm.	
<i>D. purpurea</i> (PP)	5.85	2.47	42.20	14.50
<i>D. ambigua</i> (AA)	4.17	2.36	56.50	9.80
<i>F₁</i> (PA)	4.90	2.60	53.00	12.74
<i>D. mertonensis</i> (PPAA)	5.99	3.34	56.00	20.00
Triploids (PPA)	6.07	2.88	47.40	17.50

Taking the cube of the geometric mean of length and breadth as a measure of bulk we find the proportion of tetraploid to diploid to be

89.4 to 45.4 or 1.97 to 1. This result agrees with the principle (generally applicable in the flowering plants and shown in *Drosophila*) that in the absence of segregation tetraploids or their organs are twice the size of the diploids from which they arose, or their corresponding organs.

NOTE

Haase-Bessell (1916) crossed *D. purpurea* with several different species of *Digitalis*, and among the always sterile hybrid seedlings usually found a varying number, even up to 70 per cent. of pure *D. purpurea*. She assumed that these were of parthenogenetic origin, and devoted several pages to a discussion on parthenogenesis.

We have not made any special study of this subject, but have also found among our F_1 seedlings a certain number of pure *D. purpurea*. But if the pollen used is well protected from contamination by covering not only the emasculated flowers used for the female parent, but also by covering from an early stage of development the flowers used as a source of pollen, the number of such seedlings is greatly reduced, as will be seen from the following figures:

	Seed- lings	Inter- med	<i>D. pur- purea</i>	%
3 crosses, flowers of male parent not covered	132	96	36	27
4 crosses, flowers covered	205	199	6	3

It seems to us more probable that it is merely a matter of protecting the pollen. Even when this is done it is always possible that a few stray pollen grains from *D. purpurea* flowering in the vicinity may fall on the stigma, and such stray grains would be given the preference by the ♀ *purpurea*. We cannot find it mentioned in Haase-Bessell's communication that she took any steps to protect the pollen of the male parent from contamination by insects coming from other plants. Parthenogenesis in the instance in which it has occurred in our experiments on the other hand is recognisable in its results both genetically and cytologically.

SUMMARY

The tetraploid giant hybrid between *Digitalis purpurea* and *D. ambigua* has bred true for five generations. It can be crossed back to the parental species only with difficulty and the triploid progeny are almost entirely sterile. It therefore has the essential characteristics of a new species and is named *Digitalis mertonensis* ($2n = 112$).

It has yielded a diploid parthenogenetic seedling which is sterile and resembles the original hybrid morphologically and in its chromosome behaviour.

The triploid back-cross to *D. purpurea* gives a variety of new types with unbalanced chromosome complements. Their form and variation is a proof of the qualitative differentiation of the chromosomes.

The morphological characteristics of the hybrid and its derivatives are described. In the balanced forms the influence of each species is as usual proportionate to the number of its chromosome sets present.

D. mertonensis has yielded a hybrid with *D. obscura*, having 112 chromosomes.

The fertility and sterility of tetraploid and diploid forms is shown to agree with the rule, earlier suggested, that there is an inverse correlation between the fertility of diploids and that of the tetraploids to which they give rise.

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EXPLANATION OF PLATES

PLATE VI. Leaves ($\frac{1}{3}$ natural size)

- Fig. 1. *D. purpurea* (diploid).
- Fig. 2. *D. ambigua* (diploid).
- Fig. 3. *D. purpurea* \times *D. ambigua* F_1 (diploid).
- Fig. 4. *D. mertonensis* F_2 (tetraploid).
- Fig. 5. *D. mertonensis* \times *D. purpurea* (triploid).
- Fig. 6. *D. mertonensis* \times *D. ambigua* (triploid).

PLATE VII

- Fig. 1. Microphotographs of metaphase plates from the root-tips. Top: the parthenogenetic seedling with 56 chromosomes ($F_2 \times D. ambigua$). Bottom: F_4 seedling with 112 chromosomes ($\times ca. 900$).
- Fig. 2. *D. mertonensis* \times *D. purpurea*. Plant nine months from sowing. Normal growth in centre, contrasting with the two dwarfs. $\frac{1}{3}$ natural size.
- Fig. 3. Microphotographs of marginal calyx hairs. (a), *Digitalis purpurea*; (b), *D. ambigua*; (c), (d), F_1 hybrid; (e), $F_2 \times D. purpurea$ (PPA); (f), F_2 (PPA).

PLATE VIII. Wash drawings of flowers

- Fig. 1. *D. purpurea*.
- Fig. 2. *D. ambigua*.
- Fig. 3. *D. purpurea* \times *D. ambigua* (F_1).
- Fig. 4. *D. mertonensis* (F_2).
- Fig. 5. *D. mertonensis* \times *D. purpurea*.
- Fig. 6. *D. mertonensis* \times *D. ambigua*.

For the photographs and wash drawings we are indebted to Mr N. K. Gould and Mr A. C. Wise, both of the R.H.S. Gardens at Wisley.

1

2



a

b



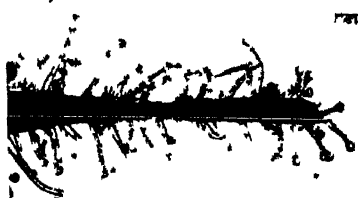
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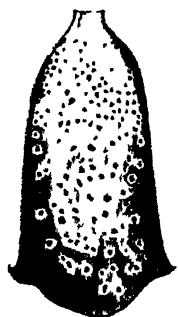
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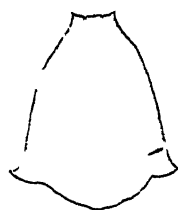


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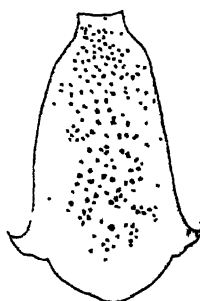
BLUNT AND DARLINGTON—CROSSES BETWEEN *DIGITALIS*
PURPUREA AND *DIGITALIS AMBIGUA*



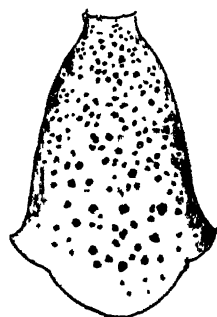
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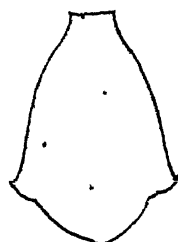
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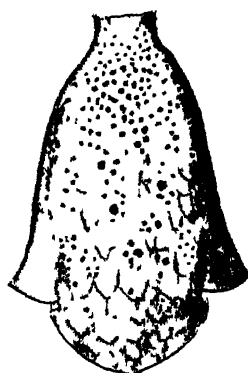
3 Fl. PA



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BUXTON AND DARLINGTON—CROSSES BETWEEN *DIGITALIS PURPUREA* AND *DIGITALIS AMBIGUA*

A FOSSIL CYATHEOID STEM FROM MOUNT ELGON, EAST AFRICA

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(With Plates IX and X and 2 figures in the text)

I. INTRODUCTION

THE fossil here described was obtained from a volcanic agglomerate near Butandiga, on Mount Elgon, British East Africa, at a height of approximately 7000 ft.; it was presented to the British Museum (Natural History) by E. J. Wayland, Esq., Director of the Geological Survey of Uganda, and it is now included in the collections of the Geological Department of the Museum under the registration letter and number "v. 20739."

The agglomerate in which the fossil was found is apparently of late Tertiary age (Ödman(4); see also Wayland(12), p. 37; (13), p. 40); further notes on this point will be contributed in a forthcoming paper on "Some Fossil Dicotyledonous Woods from Mount Elgon, East Africa."

The fossil is a short piece, somewhat over a foot in length, of what is evidently a decorticated tree fern stem, neither leaf-bases nor external roots being present. The stem is enclosed in a semi-crystalline lava-matrix, more or less broken away at one side, and its fossilisation medium is highly crystalline (Wayland(12), p. 51; Udluft(11)), so that actual cell structure is obscured, though the position of the larger masses, at least, of the woody tissues is clearly marked; since only the gross structure is determinate, the specimen has not been sectioned for microscopic examination.

The portion of the whole stem described in the following pages is a block, half an inch in thickness; both its surfaces present transverse sections of the stem, which, in its decorticated condition, is from $3\frac{1}{4}$ to $3\frac{1}{2}$ in. in diameter. The structure of this block appears to be perfectly representative of that of the stem as a whole.

Both surfaces of the block are slightly polished, and photographs of them have been taken under strong reflected light, after oiling to intensify the definition of the areas representing the woody tissues (Pl. IX).

II. DESCRIPTION OF THE STEM

1. *The meristeles*

On both surfaces of the block, nine meristeles—seven of which are U-shaped, and two broadly W-shaped—are clearly shown, each by a narrow band representing its more compact central tissues; the other tissues are not distinguishable. The presence and position of a sclerenchymatous sheath accompanying each meristele is slightly and irregularly indicated by a dark coloration of the crystalline substance of the block¹ (Pl. IX, fig. 1, Pl. X, fig. 3, *ms* and *sc*).

2. *Phyllotaxy*

The phyllotaxy is high and irregular, for the nine gaps between the meristeles and the middle points of the two W-shaped meristeles indicate the occurrence of eleven rows of leaves (cf. Ogura's figures of *Cyathea spinulosa* (5), p. 158, Fig. 1, and p. 313, Fig. 72). This high phyllotaxy may signify that the fossil stem portion under consideration was derived from a position fairly high up in the original "trunk" (cf. *C. spinulosa* (5), p. 310, Fig. 71).

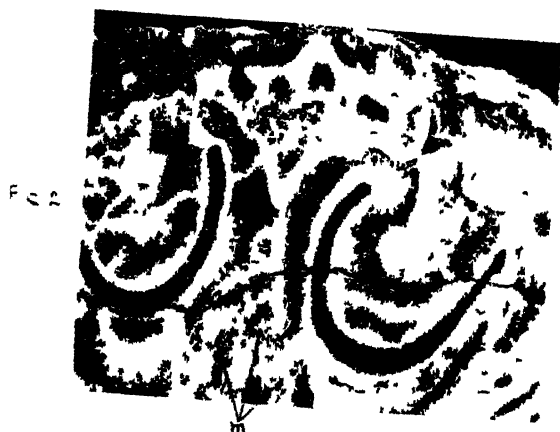
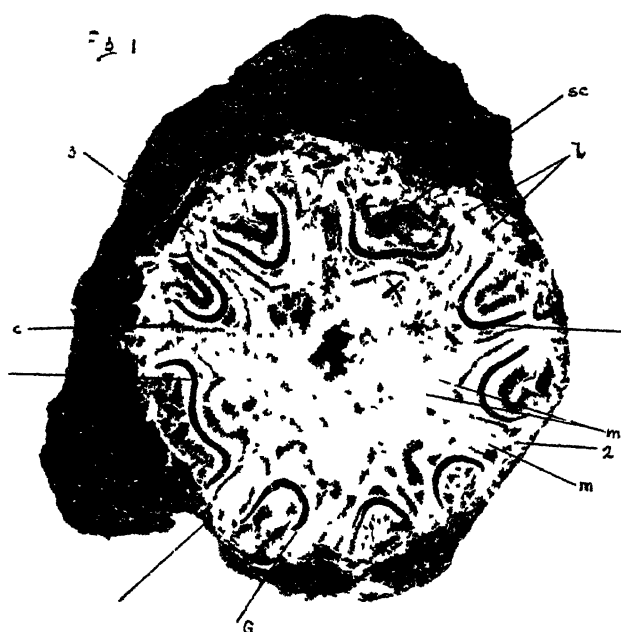
3. *Leaf-gaps and leaf-traces*

The margins of the meristeles *all turn outwards* to the cortex at the leaf-gaps (Pl. IX, fig. 1), and from them small separate strands forming the composite leaf-traces are detached, apparently in pairs, one member of each pair on each side of a leaf-gap, and one a little in advance of the other, as in recent species of the Cyatheaceae. Pl. IX, fig. 1, shows a pair of leaf-strands, *l*, in the gap immediately to the right of the meristele marked X; one of the meristeles shows preparation for the separation of a member of another pair. Text-fig. 1 A represents this case on a larger scale; while in Pl. IX, fig. 2, a leaf-gap with two pairs of leaf-strands is given.

In one or two instances there are evidences at a meristele margin of a grooving, such as that described by Ogura in the case of *Cyathea spinulosa*, where it is preparatory to the separation of a leaf-strand ((5), p. 179, Fig. 14. In Pl. IX, fig. 1, grooving may be seen with a lens at G).

In one case (Pl. IX, fig. 1, Pl. X, fig. 3) a meristele margin shows a Y-shaped structure, while just beyond the margin of the meristele

¹ Cf. the imperfect preservation of the sclerenchymatous sheaths of the meristeles in Ogura's stem *Cyathocaulis* ((6), p. 357, Figs. 18-21).



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at the other side of the leaf-gap is a series of five small separate strands arranged roughly in the shape of the two arms of a Y; their arrangement suggests that they were derived by the breaking up of a forked structure similar to that on the other side of the gap, after the separation, as a whole, of this structure from the meristele margin. It is interesting to note that Ogura has pointed out, in the case of *Cyathea spinulosa*, that two or three strands—particularly of the upper series of the composite leaf-supply—often part from the meristele as a common strand, and subsequently separate (5), pp. 163, 164; diagrams xvii and xviii in Fig. 4 illustrate this behaviour in the last pair of the series of leaf-strands). No sclerenchyma accompanying the leaf-strands can be detected; this, however, is probably due to defective preservation.

4. Medullary bundles

The medulla of the stem contains a number of small scattered bundles, the relationship of which to the inner surface of the meristetes cannot be determined. Sclerenchyma sheaths accompanying these medullary bundles, if they originally existed, have escaped preservation; their occurrence might be expected from the fact that similar bundles of members of the Cyatheaceae described by Ogura, and other writers, possess them (cf. Ogura (5), pp. 181 *et seq.*; Bower (2), 1, p. 156, and Fig. 150; Schütze (8), p. 365, and Fig. 3; Diels, in Engler and Prantl (3), p. 116). In this connection it may be mentioned that sclerenchyma sheaths also accompany many of the medullary bundles in *Cyathocaulis naktongensis*, a Japanese fossil tree-fern (Ogura (6), pp. 358, 359, Fig. 5); while in an allied and very similar type, *Cibotiocaulis Tateiwa*, Ogura notes that the apparent absence of sheaths to the medullary bundles may be due to imperfect preservation (6), p. 368).

In one or two cases there are indications that the medullary bundles possessed a medullated protostelic structure, such as described by Ogura for those of *Cyathea spinulosa* (5), p. 182, Fig. 16).

The medullary bundles of v. 20739 are especially well seen here and there in the leaf-gaps, where they occur in pairs; Pl. X, fig. 4, shows a gap with one very clear pair, and another less clear. This paired arrangement of medullary bundles in the leaf-gaps indicates their connection with the leaf-traces, as in recent members of the Cyatheaceae, though in the fossil the actual junction of a medullary bundle with a leaf-strand-producing meristele margin is not repre-

sented on either of the two transverse surfaces of the stem¹. In one case, however, a meristele margin shows a distinct protuberance as if a medullary bundle had just fused with it (the left-hand arm of the stele marked X, Pl. IX, fig. 1), while a medullary bundle is approaching the meristele margin on the other side of the leaf-gap.

5. *Cortical bundles*

Here and there are indications that cortical bundles may be present (Pl. X, fig. 3, at *c*?); the specimen, however, is too much decorticated and too badly preserved to show whether the suspected bundles have any relationship to the leaf-strands, such as cortical bundles have been shown to possess in certain recent members of the Cyatheaceae, *Alsophila Bongardiana* and *A. latebrosa*, for example (Ogura (5), pp. 248 and 290).

6. *Root traces*

No indication of root bundles could be seen in the pith of v. 20739²; in several cases in the cortex, however, near a leaf-gap, are structures which may possibly be adventitious roots (cf. Text-fig. 1, *r*(?)).

7. *Mucilage or secretory sacs*

Preservation of the stem is not sufficiently good to show whether or not mucilage or secretory sacs were originally present, as in *Cyathea spinulosa* (Ogura (5), pp. 174, 175, Figs. 9 and 10; p. 178) and other members of the Cyatheaceae.

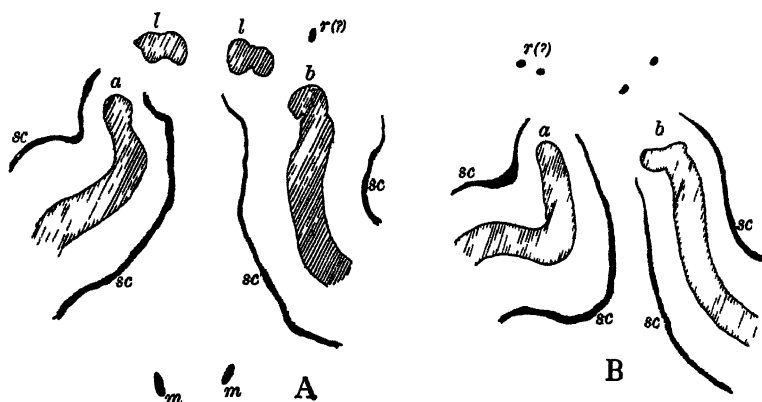
8. *The orientation of the block in the original "trunk"*

This is a difficult matter to determine from two transverse surfaces of the stem only half an inch apart; v. 20739, however, has so many points in common with the living Cyatheaceae, as described by Ogura, that it is perhaps allowable to use the behaviour of the leaf-traces and medullary bundles, and the form of the leaf-gaps, in such a type as *Cyathea spinulosa*, as a guide in determining which are the upper and lower surfaces of the fossil stem block (cf. Ogura (5), p. 163, Fig. 4).

¹ Cf. Ogura (5), p. 165, and p. 163, Fig. 4, where the connection of medullary bundles with the leaf-traces is described and figured in the case of *Cyathea spinulosa*. A similar connection is described on p. 247 for *Alsophila Bongardiana*. In *C. spinulosa*, for example, the bundles unite with the meristele margins, which subsequently separate to produce the leaf-strands; so that the medullary bundles have a definite connection with the leaf-supply. Scott (9) has also noted this connection.

² Cf. *Caulopteris Brownii* and *Cyathocaulis nakiongensis*, to which reference is made on pp. 249, 250.

In Pl. IX, fig. 1, representing surface *A* of the stem, the leaf-gap immediately to the right of the stele marked *X* shows a single pair of leaf-strands, the strands, and also the meristele margins *a* and *b*, from which they were presumably separated, being fairly close together, the margin *b* is preparing to give off another strand (see Text-fig. 1 A). In the pith, some distance from the two meristemes, a pair of medullary bundles, *m*, may be seen approaching the gap; while in the cortex outside the gap are indications of roots, *r*(?). These points are



Text-fig. 1 A (from surface *A* of the stem v 20739) A leaf-gap showing two leaf-strands (*l*), and the preparation of meristele *b* for the separation of another strand, a pair of medullary bundles (*m*) is shown approaching the gap from the pith

B (from surface *B* of the stem, but reversed to correspond with Text-fig. 1 A) The same leaf-gap without leaf-strands or medullary bundles, the meristele margins are closer than in A

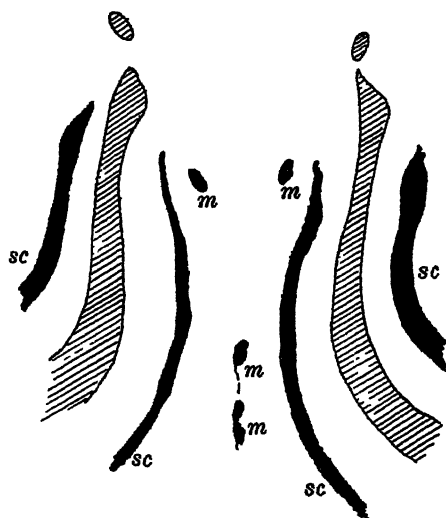
The indications of the sclerified sheath of the meristemes are shown at *sc*, and *r*(?) represents possible adventitious roots in the cortex. The magnification is 3.5 diameters

The appearance of the leaf-gap in the two sections suggests that Text-fig. 1 B represents a lower level than Text-fig. 1 A, in the departure of the vascular supply to the leaf

shown on a larger scale in Text-fig. 1 A; considered together, they indicate a position low down in the separation of the whole composite leaf-supply. On surface *B*, however, the meristele margins of the same leaf-gap are still nearer to one another, and there are no separate leaf-strands (Text-fig. 1 B); a few small scattered, more or less rounded structures, *r*(?), in the cortex outside the gap, suggest root traces, while the protuberance on meristele *b* may indicate the separation of another root trace; there are no medullary bundles in the neighbourhood of the leaf-gap.

According to Ogura's figures, Text-fig. 1 B shows a condition still lower than Text-fig. 1 A in the formation of the leaf-gap; it is, in fact, taken to be below the separation of the first pair of leaf-strands.

The surface *B* of the stem block, therefore, seems to represent a lower position in the original "trunk" than surface *A*; the examination of another leaf-gap in which the structure is sufficiently completely shown on both sides of the block supports this conclusion.



Text-fig 2. The leaf-gap on surface *B* of the stem v. 20739, corresponding to that on surface *A*, as shown in Pl. X, fig. 3. (The position of the meristele has been reversed in the diagram to correspond with their position in the photograph. As in the photograph, the magnification is three diameters)

Note that small strands have evidently just separated from the meristele margins, and that there are two pairs of medullary bundles, *m*, in the leaf-gap, which is wider than in Pl. X, fig. 3. The sclerised sheaths of the meristele are indicated as in Text-fig. 1.

A discussion of the form and structure of this leaf-gap is given in the text.

In the case, also from surface *A*, shown in Pl. X, fig. 3 (cf. Pl. IX, fig. 1, 3), the gap is narrow, and the meristele margin at one side shows a Y structure, while on the other side of the gap, just beyond the meristele margin, are five separate bundles roughly arranged in the form of the arms of the Y, as previously described (pp. 242-3); in the leaf-gap a pair of medullary bundles is present. Surface *B* of the block shows the corresponding gap considerably wider, and possessing two pairs of medullary bundles, the uppermost some little distance



FIG 3



FIG 4

BANCROFT—A FOSSIL CYATHEOID STEM

from the meristele margins, from each of which strands have apparently just separated (Text-fig. 2). If the behaviour of the leaf-strands and medullary bundles of members of the Cyatheaceae is taken as a guide, the condition of this leaf-gap, as shown on surface *A*, probably represents the separation of the first members of the superior series of the whole composite leaf-supply, after the fusion of a pair of medullary bundles with the meristele margins¹ (as indicated by the Y-shape of the one meristele margin, and the arrangement of the five strands beyond the other); the narrow gap represents the constriction between the lower and upper series of leaf-strands. The wider gap, and the *two* pairs of medullary bundles shown on surface *B*, represent a condition seen in the separation of the lower series of strands. It therefore appears, in this case also, that surface *B* represents a lower position in the original "trunk" than surface *A*.

III. COMPARISON OF THE STEM WITH RECENT TYPES

From the foregoing description of specimen v. 20739, it will be recognised that its structure has many points in common with that of the recent members of the Cyatheaceae, that is to say, with *Cyathea*, *Alsophila* and *Hemitelia*, according to Prof. Bower's limitation of the family, the Dicksonieae, *without* a medullary vascular system, being separated from those genera which possess such a system (2, 2, p. 265).

Like *Cyathea*, *Alsophila* and *Hemitelia*, v. 20739 has a dictyostelic structure, there being a single peripheral series of meristeles, the margins of which turn *outwards* at the leaf-gaps; as in the three recent genera, also, the vascular supply to the leaves consists of separate strands, and there is a system of medullary bundles evidently connected with the leaf-strands, since pairs of these occur in the leaf-gaps. Further, in v. 20739 there are indications of cortical bundles, such as are present in (for example) *Cyathea Imrayana* (1), p. 606, Fig. 337 B; (2), 2, p. 296, and 1, p. 156), *Alsophila Bongardiana*, *A. latebrosa*, and some forms of *Cyathea spinulosa* (5), pp. 247, 289 and 264)²; and, as in the recent Cyatheaceae as a whole, there are apparently no root bundles in the pith.

¹ The possibility should be noted that if cortical bundles are present, as suggested by the strands *c?* in Pl. X, fig. 3, the Y-shaped structure of the meristele margin may be due to the fusion with it of a cortical bundle (cf. Ogura's description of *Alsophila Bongardiana* (5), p. 248, and p. 243, Fig. 40). Surface *B* does not, however, sufficiently indicate the possibility of the fusion of cortical bundles. In any case, such fusion takes place in connection with the *superior* series of leaf-strands.

² In describing certain Japanese Cyatheaceae, Ogura has noted that some specimens of *Cyathea spinulosa* have no cortical bundles; in such cases, he refers to the stems as possessing "Cyathean dictyosteles" (5), p. 202; other

v. 20739 possesses, therefore, a typical Cyatheacean structure, and there need be no hesitation in allying it with this family. Generic assignation, however, presents difficulties, for in the present state of knowledge of *Cyathea*, *Alsophila* and *Hemitelia*, there appears to be no anatomical distinction between them; the classification of the genera depends, so far, on soral characters (Bower(2), 2, p. 310); and in the absence of associated reproductive material there is no reason why v. 20739 should be attached to one, rather than to either of the other, recent genera.

The Cyatheaceae are not, at the present day, extensively represented on the African continent (Diels, in Engler and Prantl(3), p. 118); *Cyathea* is more abundant than the other two genera, several species being found in the East African area—*C. Dregei*, for example, occurs in Uganda itself. The present distribution of *Alsophila* and *Hemitelia*, however, indicates that they may have covered a wider area in former times; and it is therefore not advisable to attach too much weight to purely presumptive evidence from the present occurrence of *Cyathea* in Uganda, and to suggest therefrom that v. 20739 represents some living or extinct species of that particular genus.

A comparison of the shape and number of meristeles of v. 20739 and of recent members of the Cyatheaceae may be made; such a comparison, however, is of little value, for while certain of Ogura's figures of *Cyathea spinulosa* (5), p. 313, Fig. 72, E and F) present a very similar appearance to Pl. IX, fig. 1, that of *Alsophila Bongardiana* (5), p. 249, Fig. 43) does also; and, on the other hand, his diagrams of *C. spinulosa* (5), pp. 310, 313, Figs. 71 and 72) indicate that the number and shape of the meristeles vary greatly at different levels in the same stem. Moreover, Ogura's work shows that the same species from different regions may present anatomical variations—variations affecting even so definite a point as the presence or absence of cortical bundles, as in *Cyathea spinulosa* (5), p. 308).

While, therefore, the structure of v. 20739 indicates that it is of Cyatheacean affinities, its closer alliance with one or other of the recent genera cannot be determined.

stems, such as those of *Alsophila Bongardiana* and *A. latebrosa*, with cortical bundles, possess, according to his terminology, "Alsophilan dictyosteles"; it appears, from (5), p. 260, paragraph 14, and also from p. 308, that the distinction between the two types lies simply in the absence or presence of cortical bundles. These terms are not used in the present discussion since they are misleading; for some species of *Cyathea*, even certain specimens of *C. spinulosa* itself (pp. 264, 265), have "Alsophilan dictyosteles," while most of the *Alsophilas* described by Ogura have "Cyathean dictyosteles" (cf. the key to the species given on p. 308).

IV. COMPARISON OF THE STEM WITH OTHER FOSSIL TYPES

Fossil stems showing certain resemblances in habit and structure to the Cyatheaceae are known from strata as early as the Jurassic (Seward(10), 2, p. 366; Ogura(6), p. 372).

The genus *Protopteris*, for example, represented by *P. Sternbergii* from the Lower Cretaceous of Bohemia, shows a dictyostelic vascular system, similar in its main features to that of *Cyathea* and *Alsophila*, although it differs from the living genera of the Cyatheaceae—and also from v. 20739—in having a leaf-trace consisting of a continuous, roughly horseshoe-shaped vascular band, with incurved ends and somewhat indented sides (Seward(10), 2, pp. 371 *et seq.*, Fig. 277).

The generic name *Caulopteris* has been applied to various stems of tree-fern habit; while Seward prefers to restrict the term to Palaeozoic stems with the internal structure of *Psaronius* (10), 2, p. 372), Lindley and Hutton applied it in the first place to tree-fern stems showing circular or oval leaf-scars, upon which the petiolar vascular tissue is represented by a U-shaped impression, the ends of the U being incurved; or, in some cases, by a closed oval ring, with a wide-open and inverted V near its upper end (cf. Seward(10), 2, p. 421). Stenzel, on the other hand, adopted the name for Mesozoic stems in which the leaf-trace consists of several separate strands (cf. (10), 2, p. 372); and Renault (7), pp. 72 *et seq.*) defined *Caulopteris* as a genus of erect, cylindrical fern stems, with elliptical leaf-scars showing a vascular arrangement similar to that of the living Cyatheaceae, with separate leaf-strands; such an arrangement is shown, for example, by *Caulopteris cyatheoides*, from the Neocomien of Austria. *Caulopteris Brownii* (Renault(7), p. 73; Pl. 8, fig. 10)¹, an English Cretaceous type, shows structure well preserved. Its meristeles are arranged in a single series; in some cases, however, their margins turn *inwards* to the pith; also roots occur in the pith, and there are no medullary bundles.

It will be apparent that the limits of the genus *Caulopteris* are too indefinite to allow of a satisfactory comparison with v. 20739; and, in any case, its best known species, *C. Brownii*, differs from the African fossil (and from the living Cyatheaceae) in various significant details: namely, the in-turning of some of the meristele margins, the presence of roots in the pith, and the absence of medullary bundles.

Alsophilina is a Cretaceous genus with separate leaf-traces; it is represented, for example, by *A. cyatheoides* (Potonié, in Engler and

¹ *C. Brownii* is named *C. Cottaeana* in the description of the plate.

Prantl(3), pp. 506, 507, Fig. 309). Little is, however, known of the types referred to this genus, and no further comparison can be established between them and v. 20739.

Ogura has described certain Japanese fossil tree ferns under the generic name of *Cyathocaulis* ((6), p. 351); this genus, represented by *C. nakdongensis* from the Upper Jurassic of Korea, is characterised by a dictyostelic structure, with a single peripheral series of meristemes, separate leaf-strands and numerous medullary bundles. These features, Ogura claims, are those of a "Cyathean dictyostele"; but the dictyostele of *Cyathocaulis* differs from the "Cyathean dictyosteles" of *Cyathea spinulosa* and *Alsophila Oguræ* as originally described and figured in his work on recent Cyatheaceae ((5), pp. 202 and 223; Figs. 1 and 22), for some of its meristeme margins, in any transverse section of the stem, turn *inwards* to the pith; this is due to the attachment of a succession of medullary bundles to the meristeme margins in the upper part of a leaf-gap, after the separation of the last leaf-strands ((6), pp. 356, 357; Text-fig. 4). This behaviour of medullary bundles has not so far been noted in living types; in this point, therefore, and also in the occurrence of roots in the pith, *Cyathocaulis* differs from both the recent Cyatheaceae (with which it is considered by Ogura to have affinities) and from v. 20739.

Cibotiocaulis Tateiwaæ, another Korean Upper Jurassic species, allied by Ogura to the Cyatheaceae ((6), pp. 364 *et seq.*), possesses the same type of stem structure as *Cyathocaulis nakdongensis*, and therefore differs from v. 20739 in the same points. It is still further removed from the African fossil by the behaviour of its leaf vascular supply, which is initiated as a continuous band; this, however, very soon breaks up into a number of separate strands, as in the recent species *Cibotium Barometz* (cf. (5), pp. 266 *et seq.*, Fig. 51).

V. CONCLUSION

From the foregoing comparisons it is evident that v. 20739 cannot be identified with any of the fossil genera hitherto described. On the other hand, although its structure is that of a typical modern Cyatheaceous fern, in the absence of associated reproductive fronds, it would be exceedingly unwise to employ a generic term suggesting a particular alliance with any one of the three living genera, as explained on p. 248.

Ogura's generic name *Cyathocaulis* would be a suitable one to apply to v. 20739, as indicating the similarity of the stem to those of the recent Cyatheaceae; since, however, the term has been used

for stems which show certain important differences of structure from both v. 20739 and the Cyatheaceae¹, it is not available in this case.

It is proposed, therefore, to institute the form-genus *Dendropteridium* for fossil fern stems of dendroid dimensions, of which, in the absence of associated vegetative and reproductive material, the exact affinities are doubtful. The specific name employed in such cases may indicate the similarity of the type under consideration to some recent type; or it may have reference to the area or horizon, or both, from which the fossil was obtained.

v. 20739 may thus be named *Dendropteridium cyatheoides*, the specific name being descriptive of the general type of stem structure exhibited by the fossil.

Specimen v. 20739 (British Museum, Natural History)

Dendropteridium cyatheoides gen. et sp. nov.

Diagnosis. A fossil stem of tree-fern type, having a typical Cyatheacean dictyostelic structure, with a single peripheral series of meristeles, the margins of which turn *outwards* to the cortex; the leaf-traces consisting of separate strands, a system of medullary bundles being apparently associated with the leaf-supply; the cortex showing indications of cortical bundles (as in some recent Cyatheaceae) and of roots; no indications of roots in the pith.

VI. SUMMARY

1. A fossil tree-fern stem from the volcanic series of Mount Elgon, East Africa, is described and figured.

2. The structure of the stem agrees with that of the recent Cyatheaceae; but in the absence of associated reproductive fronds,

¹ Ogura's generic names *Cyathocaulis* and *Cibotiocaulis* are both somewhat unfortunate. They naturally suggest that the stem structure of the fossils to which they are applied is similar to that of the Cyatheaceae (Cyatheae, according to Diels, in Engler and Prantl (3)) in the one case, and to that of *Cibotium* in the other case. As a matter of fact, as noted in the text, the two fossils possess the same type of dictyostelic stem structure, with some of the meristele margins turning outwards to the cortex, and others turning inwards to the pith; in both cases, roots occur in the pith, and there are medullary bundles; the behaviour of the medullary bundles in the upper part of the leaf-gaps determines the in-turning of the meristele margins. So far as the in-turning of the meristele margins and the occurrence of roots in the pith are concerned, both the Japanese fossils differ from the recent Cyatheaceae and from *Cibotium*; and *Cibotiocaulis* further differs from *Cibotium* in the possession of medullary bundles, the non-occurrence of which in the living genus removes it from the Cyatheaceae altogether according to Bower's definition of the family ((2), 2, cf. pp. 263-5 and 296-9). The terms *Cyathocaulis* and *Cibotiocaulis*, as used by Ogura, evidently refer chiefly to the similarity of the leaf vascular supply to that of the Cyatheaceae and that of *Cibotium* respectively.

the fossil cannot be referred to any one of the three existing genera.

3. The Mount Elgon stem does not agree in structure with any of the fossil genera so far described.

4. The institution of the form-genus *Dendropteridium* is therefore proposed for the reception of fossil tree-fern stems which cannot be definitely referred to already described genera.

5. The Mount Elgon stem is thus named *Dendropteridium cyatheoides* to indicate that it is a tree-fern stem with a structure similar to that of the Cyatheaceae.

The grateful acknowledgments and thanks of the writer are due to the Keeper of the Geological Department of the British Museum for permission to work at the collections of fossil plant material in his charge; and also to Mr W. N. Edwards, of the Geological Department, and Prof. Bower, for much helpful discussion.

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EXPLANATION OF PLATES

(Photographs)

Specimen v. 20739 (British Museum, Natural History)

Dendropteridium cyatheoides gen. et sp.nov.

PLATE IX

- Fig. 1. The transverse surface *A* of the stem, showing seven U-shaped and two W-shaped meristemes, *ms*; one of the latter is marked *X* for orientation purposes. A sclerised sheath, *sc*, accompanying each meristeme, is indicated with varying degrees of clearness; *l*, leaf-strands; *m*, medullary bundles; *G*, the grooving of a meristeme margin in preparation for the formation of a leaf-trace; *2*, the leaf-gap corresponding to that shown in fig. 2, from surface *B*; *3* and *4*, the leaf-gaps shown in Pl. X, figs. 3 and 4, respectively. ($\times 0.75$.)
- Fig. 2. A leaf-gap as it appears on surface *B* of the stem (corresponding to *2* on surface *A*, fig. 1); the leaf-supply consists, at this point, of four strands; medullary bundles, *m*, are present at the inner margin of the leaf-gap. ($\times 2.5$.)

PLATE X

- Fig. 3. Cf. *3* in Pl. IX, fig. 1. Note the Y-shaped margin of the left-hand meristeme, and the arrangement of the five separate strands beyond the margin of the right-hand meristeme; *M*, medullary bundles within the leaf-gap; *c?* are possible cortical bundles; *sc*, indications of the sclerised sheaths of the meristemes. ($\times 3$.)
- Fig. 4. Cf. *4* in Pl. IX, fig. 1. Two pairs of medullary bundles are shown in the leaf-gap. ($\times 5$.)

ANATOMY OF THE STELE OF *CYATHEA*
MEDULLARIS SW.

By H. GODWIN

(With 7 figures in the text)

FEW examples of filicinean vascular anatomy are less familiar, or for that matter less investigated, than the steles of the tree ferns. The only extensive modern publication dealing with them is that of Ogura in the *Journal of the Faculty of Science, Tokyo*, which is not easily accessible in Great Britain, nor do Ogura's researches extend beyond the Japanese and Formosan species. At the same time the desirability of having a description of Cyathean stelar anatomy readily available is shown by the fact that palaeobotanists are now beginning to describe fossil cyatheoid stems (see the preceding paper in this journal by H. Bancroft, "A fossil Cyatheoid stem from Mount Elgon, East Africa"), the structure of which becomes extremely difficult to follow and interpret in the absence of descriptions of living species. In addition to this, Ogura's are the only data which have appeared on the cyatheoid anatomy since the development of modern theories of filicinean stelar structure by Tansley and Bower, and in the light of examination of other species, views and interpretations different from his may easily suggest themselves. These considerations have led the author to revise and publish a short account of an investigation of the anatomy of the tree fern *Cyathea medullaris* Sw., which he carried out in 1921.

The single axis examined was 15 in. long and at its thickest 2-3 in. across. With the exception of the apical 2-3 in. which was hidden under a coat of blackish hairy ramenta, it was covered with an extremely hard sclerotic layer formed from the outer cortex. The leaf-scars arranged in a complex spiral upon it were protected with still harder sclerotic plates through which the remains of the leaf-trace bundles projected. The descending adventitious roots which densely enveloped the stem were found to arise only from localised portions of the axis below each leaf-scar, and when the cortex was removed they were seen to originate from the bundle system entering the abaxial arc of the leaf-trace from the main stele (Fig. 1).

The stem is dictyostelic in structure with a very evident tubular nature, the meristeles being broad and band-like, and seen as a rule

in transverse section to be separated by three leaf-gaps (Fig. 3). The chief interest of the stele centres on the medullary bundle system, which may be seen in transverse section of the adult axis as from fifty to sixty strands scattered separately through the pith. Each strand is completely surrounded by its own dark brown sheath of sclerenchyma, and a dissection shows frequent fusions with other

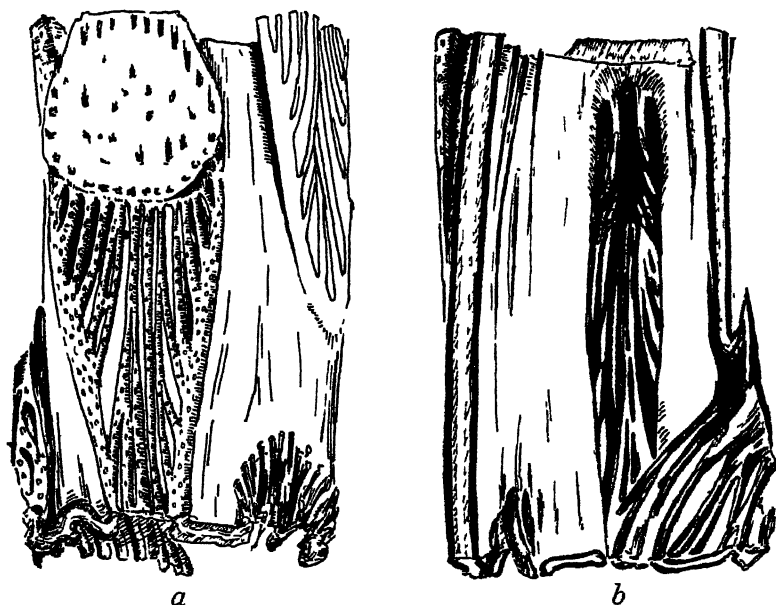


Fig. 1. *a*. Dissection showing the stelar tube after removal of the cortex. It shows the bundle system supplying the abaxial arc of the leaf-trace and bearing the scars of adventitious roots. These scars have not been drawn in the upper leaf-gap. In the lower right-hand leaf-gap the sclerotic plate of the leaf-scar has been cut away.

b shows the same dissection from the inside, with all the pith and most of the medullary bundle system removed. From the main leaf-gap all the medullary bundle supply has been cut away, but that running to the lower right-hand gap has been left.

bundles and with the main stelar tube. The cortical bundle system described as occurring in *C. imrayana*, *C. spinulosa*, *Alsophila bongardiana* and *A. latebrosa* is not present.

The leaf-scar shows the arrangement of the bundles of the leaf-trace, which is interpretable as a gutter-shaped unit with inturned margins and a deep fold running laterally down each side (Fig. 2 C). It is seen in transverse section to consist of the following parts: three

peripheral bundle arcs of which two are adaxial and one abaxial, two bundle series representing the inturned margins of the trace, and the two superior (adaxial) and the two inferior (abaxial) series forming the lateral tucks (Fig. 2 B). These terms seem to the author preferable to those of Ogura who does not speak of the inturned margins of the leaf-trace, but groups these strands with the superior series of the lateral fold as the "superior series" and so separates them from the inferior series of the lateral fold which constitute the "inferior series."

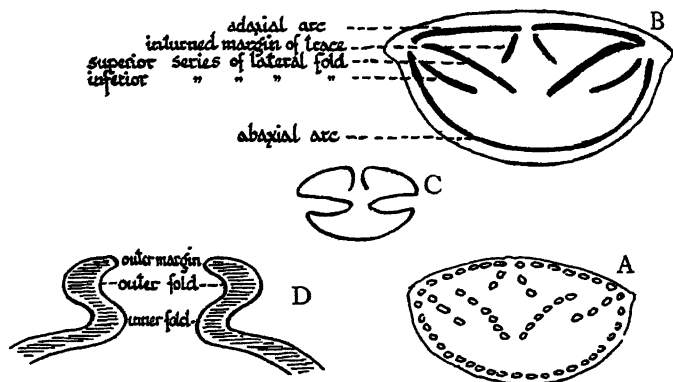


Fig. 2. Diagrams showing the nomenclature applied to the leaf-trace and leaf-gap systems. A, transverse section through the petiole showing separate petiolar strands. B, diagram to show the terms applied to different groups of these strands. C, diagram to show the concept underlying the terminology, namely that the system can be regarded as a gutter-shaped trace with inturned margins and two deep lateral folds. D, transverse section through a leaf-gap showing the folded margin and the terms employed in connection with it.

The leaf-trace is symmetrical about a vertical line. The shape of the leaf-gap is conveyed by Trécul's term "an open button hole," but the margins of the leaf-gap are bent outwards in a double fold so that in a cross-section the leaf-gap margins are curved into S shapes, of which one may speak of the inner (convex) fold, the outer (concave) fold, and the outer margin (Fig. 2 D). Strictly speaking this folded margin of solid meristele only occupies that part of the leaf-gap which lies below the lateral folds in the leaf-scar: this amounts to about five-sixths the total length of the gap and seems related to the bundle supply to the abaxial arc of the leaf-trace, for above the point where the last of these bundles has gone into the leaf the gap has an unfolded margin.

From the dissection shown in Fig. 1 it was obvious at once that the abaxial arc of the leaf-trace arises from the outer margins of the leaf-gap. This abaxial arc bundle system consists of anastomosing bundles which form a lattice over the leaf-gap before they enter the leaf-base. These are the bundles bearing the adventitious roots.

To determine the course of the other bundles of the leaf-trace, leaf-bases were dissected from both back and front (Fig. 4), and these showed that the inferior series of the lateral fold of the leaf-trace is connected definitely with the *inner* fold of the leaf-gap. The superior series is connected with the inferior series, with the adaxial

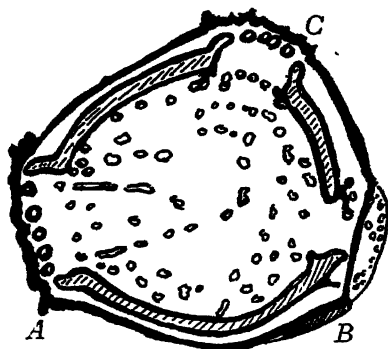


Fig. 3. Representative transverse section of stele passing through three leaf-gaps, showing three band-like meristemes, a large number of medullary bundles, and the hard outer cortex. Leaf-gap C is cut low down and the strands going to the abaxial arc of the leaf-trace are few: gap A is cut higher up and these strands are more numerous: gap B is cut near the top and the abaxial leaf-trace strands have all been given off. C shows the origin of medullary bundles (running upwards) from the inner fold of the leaf-gap margin and B shows the medullary bundle system which supplies this gap fusing laterally with the leaf-gap margin.

arc, and with the outer margin of the leaf-gap. All this part of the bundle system is connected up in a plane approximately parallel with the leaf-scar and just below it, the whole forming a sort of plate fusing laterally with the leaf-gap margin and having the form of a broad horseshoe with the central slit open towards the middle of the abaxial arc (Fig. 4 c). The dissection examined from the back showed that a number of medullary bundles, anastomosing in places, converge at the leaf-gap, and, there condensing into one or two main strands, fuse with the inner fold of the leaf-gap margin forming the vascular plate before mentioned, from which the various leaf-trace bundles separate. When the medullary bundle system entering the

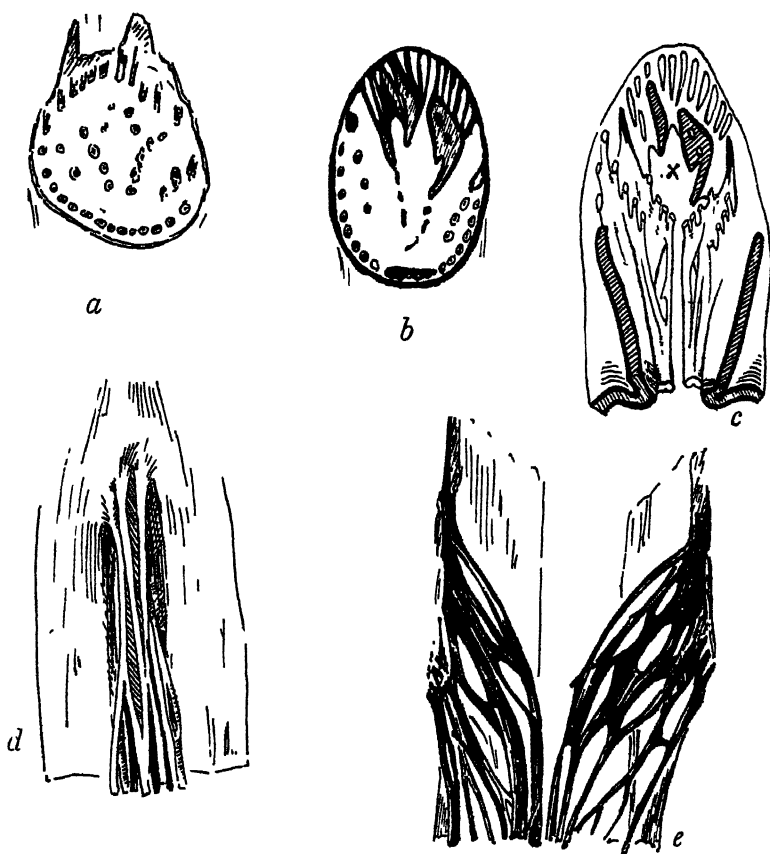


Fig. 4. Drawings of a dissection of a node. *a*, leaf-base. *b*, the same with the sclerotic leaf-scar cut away. The plane of the section shows the bundles of the abaxial arc and most of the bundles of the lateral fold still free; the bundles of the adaxial arcs are no longer visible, having fused with the main solenostele at the upper margin of the leaf-gap; the upper bundles of the superior series of the lateral fold and the bundles of the inturned margin of the trace have fused and form a vascular plate, the cut surface of which is shown shaded. *c*, the node as in *b*, but dissected from the front, showing the vascular plate which is fused laterally with the leaf-gap margins and which bears the strands of the lateral folds and inturned margins of the leaf-trace. From the centre of the leaf-gap, marked X in the diagram, there has been purposely omitted all the bundle system travelling back from the leaf-base into the medulla: this system is shown in *d* and *e*. *d*, the node dissected from the back showing medullary bundles entering the leaf-gap (other medullary bundles have been cut away). *e*, the medullary bundle system supplying the gap cut longitudinally and opened.

leaf-gap was split longitudinally the two halves were seen each to consist of a net of vascular strands, or a perforated band of vascular tissue fusing with the main cylinder where it enters the leaf-base and becoming more finely divided as it passes into the main axis (Fig. 4 e).

The drawings of Fig. 5 are intended to convey in a semi-diagrammatic manner the passing of a leaf-trace from the stem. For clearness the leaf-trace system has been represented as three solid bands instead of separate bundles; this solidification has been made also for the abaxial bundles before entering the leaf, and in B other leaf-gaps in the stem have not been drawn. Both these reconstructions show how evident is the connection between the medullary bundle system and the inner fold of the leaf-gap.

When portions of the medullary bundle system were dissected out it was found that the medullary bundles always arise from the main stelar cylinder at the level of the lower parts of the leaf-gap, and more abundantly near the gap (i.e. to the inner fold of the leaf-gap margin) than away from it (Fig. 6). The bundles arise, then, mainly from the inner fold of the leaf-gap margin and by the lower part of the leaf-gap; they pass up the stem and for a time neither branch nor anastomose; they bend round the medullary bundles entering the leaf-gap beside which they arose, and, without fusing with these at all, pass on up the stem. One can usually find, however, one bundle arising from the lowest part of the leaf-gap, which turns and fuses with the lateral edge of the strip of medullary bundles entering the same leaf-gap. With this exception the medullary bundles pass the leaf-gap by which they arise and move up to the middle of the stem, where they branch and anastomose to form a loose meshing from which they pass out into the leaf-bases, several roughly arranging themselves as they do so into two more or less radial strips. It is probable that of the bundles arising from beside any one leaf-gap, half goes to each of the two leaf-gaps next above. In saying this one must remember that the individuality of the bundles is partly lost whilst they occupy the middle of the stem; there they fuse with other bundles, so that no one bundle can be said to do any one thing from the time that it first branches or fuses with another.

The course of the medullary bundles could also be described by saying that upon leaving the leaf-base they enter the stem and move obliquely to the middle of it; they descend the stem and may anastomose with similar bundles, or fuse again with the main stelar tube on the fold of the same leaf-gap or, more often, of the leaf-gaps next below.

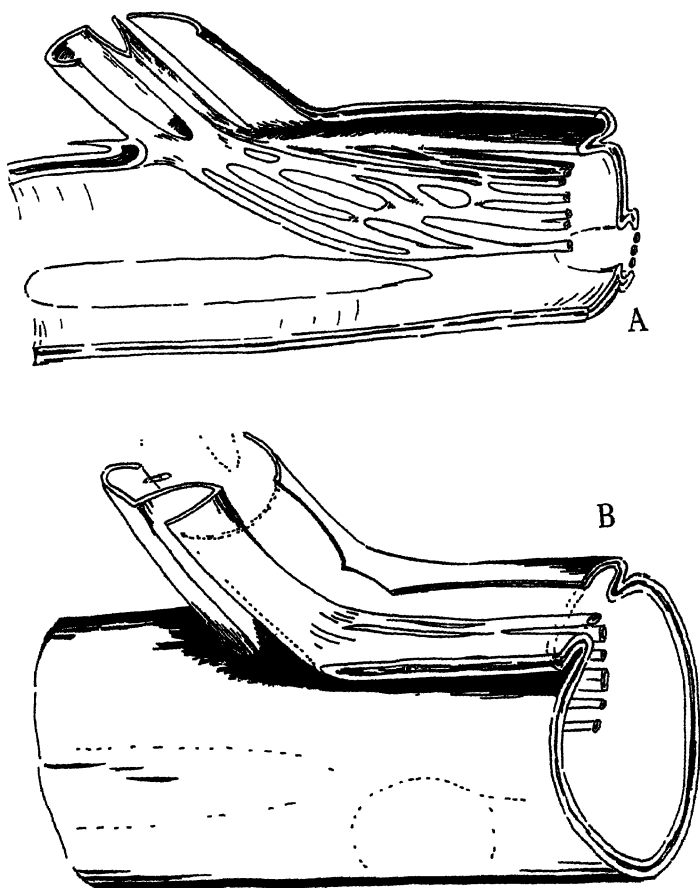


Fig 5 Diagrams to illustrate the essential features of the connection of the medullary system supplying a leaf-gap with the margins of the gap and with the different components of the leaf-trace system. For the sake of clarity only half this medullary system has been shown and no other medullary strands; in addition the leaf-trace system has been shown as three solid bands, (a) the abaxial arc, (b) one adaxial arc with the intumed margins and lateral fold on the right of the trace, (c) the corresponding group on the left of the trace.

The medullary bundle system approaches the gap (principally) as two radial series of bundles which fuse laterally with the inner folds of the leaf-gap margins in the upper part of the leaf-gap. They condense as they reach the leaf-base and form an almost solid vascular plate in the upper part of the leaf-gap. This plate gives rise to the strands of the intumed margin and lateral folds of the leaf-trace.

The medullary bundle system of *Cyathea medullaris* is composed entirely of such bundles as these described, running these courses. The characteristic features of their behaviour are indicated in transverse sections of the stem, which naturally cut the different leaf-gaps at different heights. Thus all sections across the middle of the gap (just below the leaf-scar) show both the external series of the abaxial arc and also the double series of medullary bundles coming out from the centre to fuse with the inner folds of the leaf-gap (Fig. 7 *e* and *d*). At a lower level may be seen the departure from the leaf-gap margin

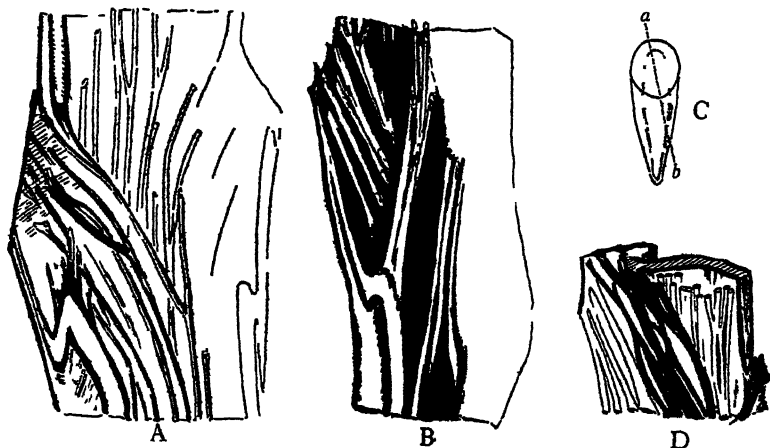


Fig. 6 Dissections on both sides (A and B) of the obliquely cut leaf-gap shown in C. Both A and B show the two systems of medullary bundles associated with each leaf-gap, i.e. one passing out to the leaf-trace, and one originating from the inner fold of the leaf-gap margin (in the lower part of the gap) and passing round the first set and up the stem. D is a dissection of part of another leaf-gap showing the two medullary bundle systems (one black and one white), and also on the extreme left a few strands of the lattice supplying the abaxial arc of the leaf-trace.

of the medullary bundles running to higher leaf-gaps (Fig. 7 *f*). At a higher level (Fig. 7 *c*) the gap is almost closed by the medullary bundles forming the vascular plate across it and giving off bundles to the middle of the leaf-scar. Higher still the vascular plate has completely closed the leaf-gap (Fig. 7 *b*), and it can be seen to be giving off bundles to the adaxial arcs and inturned margins of the leaf-trace.

Similar features may be seen in the different leaf-gaps of the stelar section shown in Fig. 3. Especially characteristic are the two radial rows of medullary bundles in the gap A (about half-way down

the leaf-gap) and the origin of medullary bundles from the inner fold of the margin in gap *B* which is evidently cut at a lower level than *A*.

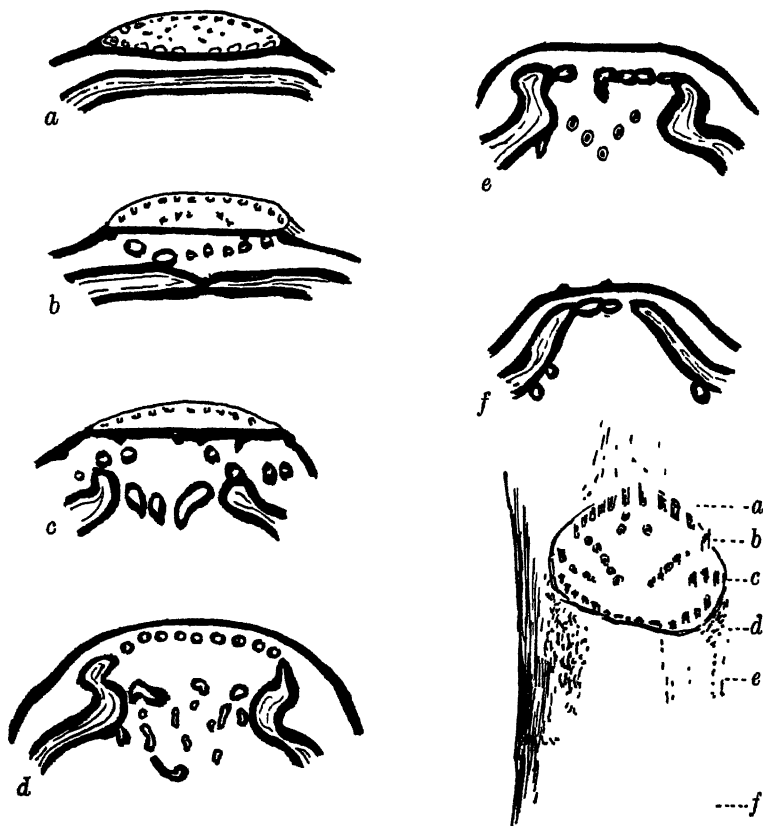


Fig. 7 A series of transverse sections through a leaf-gap at definite levels, showing in *f*, strands of one medullary system originating from the main stele, and in *e*, *d* and *c*, strands of the other medullary system supplying the leaf-trace and fusing with the leaf-gap margins. Fuller description in text.

DISCUSSION

Gwynne-Vaughan suggested that polycycly originated in the Filicales by the development of the thickened inner fold of the leaf-gap margins and this, it was suggested by Bower, would afford a reasonable basis for explaining cyatheoid anatomy. So far, however, as the author is aware, the present paper demonstrates a far more intimate relation of medullary bundles to the leaf-gap margins than has been

shown before. According to Ogura in the Japanese tree ferns the medullary bundles, though connecting with the leaf-gap margin at which they leave the stem as petiolar strands, make contact with the stele at no other point, and in a downwards direction anastomose with other bundles and (with rare exceptions) merely end blindly in the pith.

Bower has described the cyathean petiolar structure as of the most highly evolved type, and has described the inturned margins and the lateral folds of the leaf-trace as modifications of an original gutter-shaped trace, evolved in relation to increasing leaf size, increasing leaf activity and the necessity for increased conduction.

It would probably appear that the features most recently evolved are the medullary system in the stele and the lateral folds and inturned margins in the leaf-trace. On this account it is of special interest to find at the leaf-gaps the clearest communication between just these three systems. This close relationship is readily appreciable in Fig. 5. It corresponds to Fig. 11 in Tansley and Lulham's paper on *Matonia*, in which the inturned leaf-trace margin is shown as a direct continuation of the internal steles of the *Matonia pectinata* polycyclic solenostele and with the results obtained by the same authors for *Pteris aquilina*, in which fern lateral folds are also developed in the petiolar trace and connect directly at the node with the *internal* of the two concentric dictyosteles of the rhizome. The arrangement of the bundles in the leaf-trace in no way corresponds with that of the medullary bundles as they pass from the leaf-base to the centre of the stem, the bundles of the leaf-trace are kidney-shaped and those of the medullary system are circular in section, and the vascular plate is interposed between the two systems. Nevertheless they connect up unmistakably with one another and so lend support to the suggestion that they arose as correlated structures. Ogura's figures and descriptions of the ontogeny of the medullary bundle system of the Japanese Cyatheaceae show unmistakably also that these bundles always first appear in connection with those parts of the leaf-trace which we have called the inturned margins and lateral folds.

It should be pointed out in conclusion that though in *C. medullaris* itself it appears that the medullary bundle system may well have originated from internal projections of the stele connecting up the margins of one leaf-gap with those above and below, it remains to bring into line with such a hypothesis the numerous cases described by Ogura and other workers both for living and fossil forms, in which

the medullary bundles do appear to arise individually and separately in the pith, or in other words appear to end blindly in the pith in a downwards direction.

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VARIATIONS IN MEGASPORE NUMBER IN *BOTHRDENDRON MUNDUM*

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(With 5 figures in the text)

IT is a familiar fact that, whilst the number of spores in the megasporangia of most species of *Selaginella* is normally four, some degree of variation occurs. Recently Duerden⁽¹⁾ has brought together the records of such variation and extended these by systematic observations on a number of species. As a result of his investigations he records increase in megaspore numbers in five species, reduction in spore numbers in six species, and inequality of spore size in seventeen species. It will be obvious that data of so complete a character are not available in the case of fossil lycopods, but an examination of the megasporangia of *Bothrodendron mundum* in the Williamson and Scott Collections¹ and in that of University College, Nottingham, has shown that some variation exists in this species also.

The megasporangia of *B. mundum*, like those of *Selaginella*, normally contain four equal spores and isolated spores are common objects in sections of coal-balls. These spores are approximately isodiametric with three large flattened facets marking the area of contact with the remaining spores of the tetrad. The point of junction of the three facets is prolonged into a relatively large hollow beak which is somewhat pear-shaped with the narrow end forming the point of attachment to the spore. The wall of that part of the spore which is not in the area of contact with its fellows is rounded. The exine is extremely thick and is provided with numerous spines which bifurcate distally and may fork two or three times. In some specimens the intine and megaspore membrane may also be detected.

Intact sporangia are less common, whilst sections of strobili are rare and, where known, are somewhat crushed. The more complete specimens have been described by Watson⁽⁹⁾ and a figure of an isolated megasporangium is given in Scott's *Studies* ⁽¹⁶⁾, p. 181.

The examples recorded in the present paper show considerable variety and will be described separately.

¹ Both these collections are now in the Department of Geology, British Museum (Natural History).

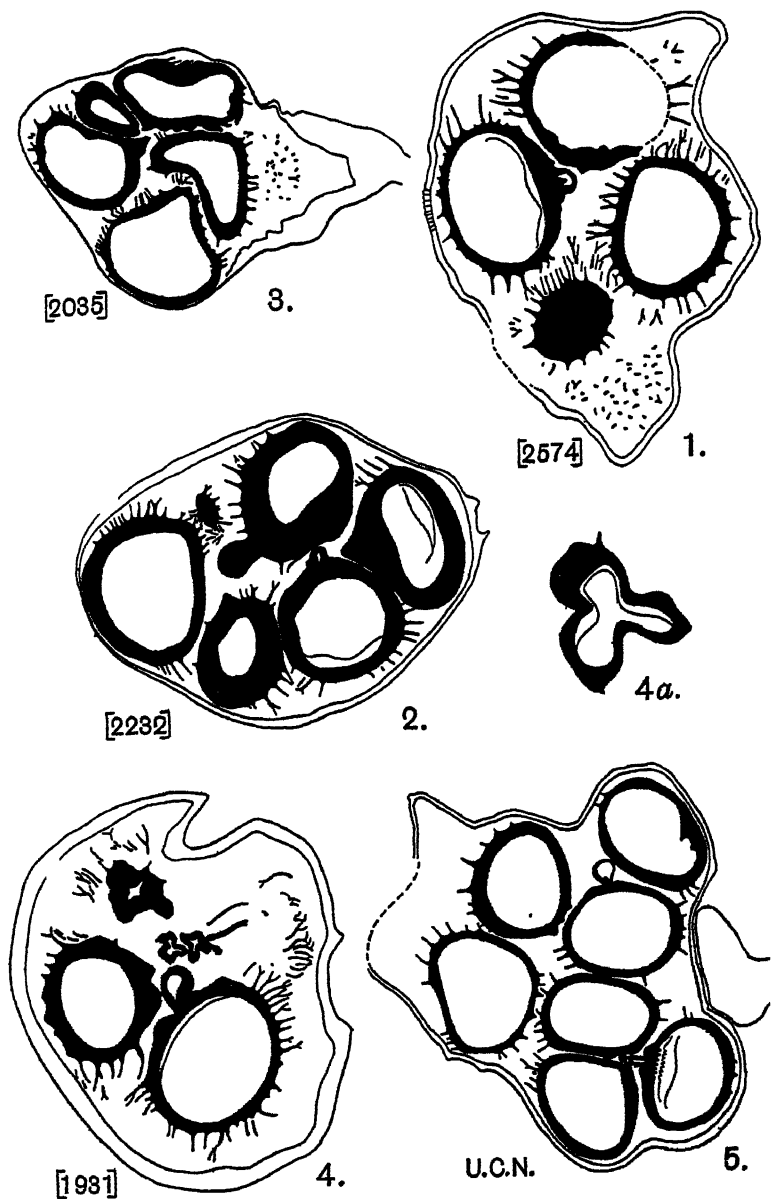
The first (Scott Coll. No. 2574) contains four clearly defined spores. One of these is cut in practically median section and shows the base of the beak, two others are cut somewhat tangentially, while the fourth is represented solely by a section of the thick spinous outer wall. In addition to these, however, one portion of the sporangium is occupied by a cluster of sections across the spines of what, it is reasonable to assume, is a fifth spore (Fig. 1). The view that these spine sections represent a fifth spore is supported by their grouping and by the outline of the group as a whole. The fact that isolated spines are not present in large numbers in intact sporangia and where occurring are not cut across so uniformly as in the example figured also lends support to their being interpreted as marking the site of a fifth spore (cf. Fig. 4).

The second specimen (Scott Coll. No. 2232) shows five undoubted spores and what appears to be a portion of the wall of another. Four of the spore sections are about median, but the fifth is distinctly tangential and gives an appearance of formidable solidity to the outer wall. The small isolated oval segment of wall with its spine bases is probably a sixth spore of which only the margin is in the plane of section (Fig. 2).

The third specimen (Scott Coll. No. 2035), like the previous one, also shows five clearly defined spores and what is probably a sixth represented in this instance by a group of spines. Three of the spores in this specimen show some degree of distortion, an unusual feature in *Bothrodendron* and one possibly indicating an immature condition. Of the remaining two, one is normal in appearance and the fifth is cut very tangentially, is pear-shaped, and seems to have undergone some compression (Fig. 3).

The fourth specimen (Scott Coll. No. 1931), which again shows five spores, is, in some respects, of outstanding interest. Of the spores present one is shown in almost perfectly median section, one is cut somewhat tangentially, and a third very tangentially in a plane at right angles to and immediately below the base of the beak. In addition to these there are two other much smaller trilobed objects which are evidently abortive spores (Fig. 4). One bears a characteristic bifid spine and the bases of two such spines as well as the inner spore membrane are clearly seen on the other (Fig. 4 a).

The remaining specimen, which is in the Nottingham (University College) Collection, appears to be a somewhat tangential section and includes part of the obliquely cut sporophyll. It contains seven fine megaspores, three of which clearly belong to one tetrad. The rela-



Figs. 1-5. Sections of megasporangia of *Bothrodendron mundum* (all, except Fig. 4 a, $\times 45$). The figures in square brackets are the numbers of the slides in the Scott Collection in which the examples occur.

tions of the remaining spores to each other are indeterminable, but it seems obvious that there must have been at least two complete spore tetrads, if not more, within the one sporangium. These spores provide the only instance I have observed in which the increase in megaspore number is accompanied by a well-defined uniform reduction in the size of the individual spores¹. One spore, shown lying near the bottom left-hand margin of the sporangium in Fig. 5, is remarkable for the peculiar sculpturing of the inner surface of the exine at its apex. This sculpturing consists of a series of square-topped ridges alternating with similar grooves and is repeated in the adjacent intine. Somewhat similar characters are occasionally shown by megaspores in normal sporangia, but the condition is sufficiently rare to be worthy of record.

DISCUSSION

It is generally conceded that the homosporous condition is primitive and the heterosporous one derived. Among the Pteridophyta, however, heterospory seems to have made little headway except among the Lycopodiales. Of the Equisetales *Calamostachys casheana*, still possessing a large number of spores in its megasporangia, is an isolated example, whilst the Psilotales and Sphenophyllales are, as far as we know, entirely homosporous although a single strobilus of *Sphenophyllum* described by Thoday (8) is suggestive of incipient heterospory. Of the Filicales only two small families, the Salviniaceae and Marsiliaceae, possess microspores and megaspores.

The Lycopodiales offer a striking contrast, since they are predominantly heterosporous and furnish a series of sporangial types illustrating progressive specialisation. Of these *Lepidostrobus* among the fossil forms and *Isoetes* among the existing ones both show a relatively large number of spores in each megasporangium. Some species of *Selaginellites* (e.g. *S. elongatus*, *S. suissei*) show a reduction in megasporangial spore output compared with these, whilst others, such as *S. primaevus*, resemble *Bothrodendron mundum* and most species of *Selaginella* in that only a single tetrad of functional megaspores appears to have been produced (2, 5, 6, 7, 10).

There is, unfortunately, no structural material of any other species of *Bothrodendron*, but *B. kiltorkense* from the Upper Devonian

¹ Measurements of the maximum diameter of twenty normal megaspores in the plane at right angles to the beak gave an average of 0.110 cm. with a range of from 0.125 cm. to 0.100 cm. The average diameter of the smaller spores is 0.082 cm.

of Ireland is described as possessing about twenty megaspores (3, 4), and is thus comparable with *Selaginellites suissei*. It should be borne in mind, however, that *Bothrodendron* is not necessarily a natural genus, and we have little beyond the external stem characters to go upon in assigning the Irish specimens to it. The exact relationships of *B. kiltorkense* to *B. mundum* do not affect the general conclusion that homospority preceded heterospority, nor that the evolutionary trend in the megasporangium has been towards progressively greater reduction in spore output. On this view the examples of *Bothrodendron mundum* megasporangia, showing evidence of the maturation of more than one spore tetrad, are to be regarded, like similar examples in *Selaginella*, as partial reversions to a more primitive condition.

Facilities for studying the specimens in the Scott Collection were granted by the Keeper of the Department of Geology, Natural History Museum, South Kensington, to whom grateful acknowledgments are due.

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ON THE GENERA *CLEPSYDROPSIS* AND *CLADOXYLON* OF UNGER, AND ON A NEW GENUS *AUSTROCLEPSIS*

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(With 1 figure in the text)

IN a paper recently published¹ I have briefly expressed the view that the Australian zygoterid generally known under the name *Clepsydropsis australis* is not a *Clepsydropsis*, in spite of its petiolar structure, and should be transferred to a new genus.

The object of the present communication is to state my full reasons for this view.

A REVIEW OF THE GENUS *CLEPSYDROPSIS* UNGER

The grounds for creating a new genus for the Australian zygoterid will be best appreciated if we review briefly the history of the genus *Clepsydropsis*. The name *Clepsydropsis* was proposed by Unger² for certain fragmentary axes, believed to be fern petioles, of which the distinctive feature was the form of the vascular bundle as seen in transverse section. It is important to emphasise that the genus was an artificial one, no other parts of the plant being known; the affinities were therefore quite uncertain³.

About the parent stem there has been a good deal of speculation, but nothing is yet definitely known. Prof. Paul Bertrand once suggested that *Clepsydropsis* rachises were borne upon stems of the *Cladoxylon* type⁴, with which they were found associated at Saalfeld in large numbers. This view, however, was severely criticised by Solms-Laubach⁵ and was subsequently withdrawn⁶. Indeed, Bertrand was able to confirm Solms-Laubach's observation that the petioles of *Cladoxylon* were not of the *Clepsydropsis* type, but were large dorsiventrally constructed organs with a compound leaf-trace: these organs

¹ Sahni (1930), p. 466.

² Unger (1856), p. 79.

³ Unger placed *Clepsydropsis*, like several other "genera" founded on fragments of supposed fern petioles, in an artificial group, the Rhachiopterideae of Corda.

⁴ Bertrand (1908); (1911 a), p. 250; (1911 b).

⁵ Solms-Laubach (1910), pp. 540-1; see also Seward (1917), pp. 204-5.

⁶ Bertrand (1913), pp. 918-19; (1914).

were found in association with the *Cladoxylons* and were known under the provisional name *Hierogramma*. Organs of the *Hierogramma* type were, in fact, found attached to *Cladoxylon* stems¹. *Clepsydropsis* was thus again left alone, as a rachis of unknown attribution, and till now no definite clue as to the parent plant has been found.

It is true that the structure of the foliar bundle had already earned for this genus an undisputed place among the Zygopterideae, and this seemed to find support from the recent discovery that in the Australian zygopterid petioles with a *Clepsydropsis*-like bundle were borne upon stems of the well-known *Ankyropteris Grayi* type². This discovery led to the belief that the Thuringian rachises must also have been borne upon stems of the same kind. Indeed, it was thought that the problem as to the stem of *Clepsydropsis* had at last been solved. However, there was no real ground for referring the Australian plant to Unger's genus, for, as I have recently shown, the clepsydroid foliar bundle is by itself no proof of generic identity³.

In the past, of course, the name *Clepsydropsis*, based on the sole criterion of the foliar bundle, has been employed rather indiscriminately. Stenzel⁴ employed it as a sub-genus of Corda's *Asterochlaena*, and he grouped under it such diverse plants as the following:

	Names as in Stenzel, 1889	Names now generally adopted	Occurrence	Age	Stem
1.	<i>Asterochlaena</i> (<i>Clepsydropsis</i>) <i>kirgisica</i> or <i>Clepsydropsis kirgisica</i>	<i>Asterochlaenopsis kirgisica</i>	W. Siberia	Probably Permian	Known
2.	<i>Asterochlaena</i> (<i>Clepsydropsis</i>) <i>antiqua</i> or <i>Clepsydropsis antiqua</i>	<i>Clepsydropsis antiqua</i>	Thuringia	Lower Carboniferous	Unknown
3.	<i>Asterochlaena</i> (? <i>Clepsydropsis</i>) <i>noeboracensis</i>	<i>Asteropteris noeboracensis</i>	Canada	Upper Devonian	Known
4.	<i>Asterochlaena</i> (<i>Clepsydropsis</i>) <i>duplex</i>	<i>Metaclepsydropsis duplex</i>	W. Europe	Carboniferous	Known

More recently I have myself inadvertently referred the Australian zygopterid to *Clepsydropsis*, having been misled by the resemblance in the foliar bundle.

The question is, are we justified in continuing to employ an old generic name, originally applied to imperfectly known fragments of obscure affinity, for more completely known plants from distant regions of the world and of different geological ages, merely because they all possess a clepsydroid bundle in their leaves?

¹ Solms-Laubach (1896), Pl. 2, fig. 13; Pl. 3, fig. 4; *ibid.* Pl. 2, fig. 10. See also Scott (1923), p. 162, Fig. 64.

² Osborn (1915); Sahni (1919); Sahni (1928).

³ Sahni (1930), pp. 465-6.

⁴ Stenzel (1889).

Of the several plants referred by Stenzel to *Clepsydropsis*, two (*Asteropteris noveboracensis* and *Metaclepsydropsis duplex*) are now universally recognised as distinct genera. As for the *Asteropteris*, Stenzel was himself doubtful about the reference to Unger's genus; the reference of the other species to *Clepsydropsis* could scarcely have been justified even on the petiolar structure alone, and Paul Bertrand recognised this fact by creating for it the genus *Metaclepsydropsis*.

The Siberian species once referred to *Clepsydropsis* was also recently transferred to a new genus, and full reasons for this were given in my paper already cited. I will now try to show that the use of the name *Clepsydropsis* cannot be justified for the Australian plant any more than for the Siberian.

I have already suggested elsewhere that if we are to avoid confusion the name *Clepsydropsis* should be restricted to the Thuringian rachises and to any clepsydroid rachises of unknown attribution which may be discovered in the future. *Failing this restriction the only logical course would be to place in the same genus both the Siberian and the Australian zygopterids, and in fact all newly discovered plants which may happen to have clepsydroid foliar bundles, regardless of any differences which they may show in their stem and leaf-trace characters.* It seems to me obvious that even with our present imperfect knowledge of the Zygopterideae a grouping so artificial as this should not be tolerated.

There can be no doubt that if we had found only the stem and the proximal part of the leaf-trace, the Australian plant would have been referred to *Ankyropteris*, as Mrs Osborn was actually inclined to do¹. It is equally certain that if we knew only the higher parts of the rachis, where the foliar bundle shows its definitive form, the plant would be assigned to *Clepsydropsis*, and this has actually been done. But knowing, as we do, that the plant combines an *Ankyropteris*-like stem with *Clepsydropsis*-like petioles, we are on the horns of a dilemma. Should we give prime importance to the stem and leaf-trace structure and call the plant an *Ankyropteris*, or should the fully formed petiolar bundle, on which the classification of the group is so largely based, have precedence? A simple expedient which I once suggested² was to combine the two genera, merging *Ankyropteris* in the older genus *Clepsydropsis*; but it has recently become apparent to me that such a course would only have caused further confusion.

¹ See Sahni (1919), pp. 82, 83.

² Sahni (1918), pp. 375-6; (1919), p. 84.

In the history of the genus *Clepsydropsis* we thus see an instructive example of the way in which reliance upon an isolated character, namely the Clepsydroid bundle, has again and again led us into difficulties.

The only alternative seems to be to create a new genus, based upon a *synthesis* of the characters. I therefore suggest for the Australian zygopterid a new generic name *Austroclepsis*. The plant would thus be renamed *Austroclepsis australis* (E. M. Osborn) Sahni comb.nov.

Austroclepsis Sahni gen.nov.

Diagnosis. Zygopterid ferns combining *Clepsydropsis*-like, *Ankyropteris*-like and *Tempskya*-like characters. The definitive form of the foliar bundle is identical with that in *Clepsydropsis*¹; the stem stele corresponds almost exactly with that of *Ank. Grayi* or *Ank. scandens*²; the leaf-trace, both at its origin and in its course through the stem cortex, also agrees mainly with that of *Ankyropteris*, except that the structure is simpler owing to the absence of an adnate axillary strand³; the repeatedly forked leaf-bearing stems are bound together by means of adventitious roots into a *Tempskya*-like false stem⁴.

Occurrence. Carboniferous of Australia. The only genus of Zygopterideae so far known from the southern hemisphere, with the possible exception of a *Botrychioxylon*-like axis epiphytic among its adventitious roots⁵.

Affinities. The nearest known ally is the Siberian genus *Asterochlaenopsis* Sahni⁶. This genus resembles *Austroclepsis* in having a *Clepsydropsis*-like petiolar bundle and a stem stele differentiated into an outer and an inner zone; but it differs from the new genus in having a simple stem, in the numerous rays of the central part of its stele, in the structure of the leaf-trace both at its origin and during its course through the stem cortex, and in the slightly abaxial points of origin of the pinna-traces which thus approach the condition in *Asterochlaena*⁷. From *Ankyropteris* the new genus differs in its *Tempskya*-like false stem, and especially in the final form of the foliar bundle, which is that of a typical *Clepsydropsis*. *Clepsydropsis* Unger is known only from its rachises. In their individual structure

¹ Sahni (1928), Pl. 4, fig. 19.

² *Loc. cit.* Pl. 4, fig. 15, etc.

³ *Loc. cit.* Pl. 3, fig. 10; Pl. 5, figs. 30, 31, 34, 35, etc.

⁴ *Loc. cit.* Pl. 1, fig. 1.

⁵ *Loc. cit.* Pl. 6, figs. 50, 51, and text, p. 25.

⁶ Sahni (1930).

⁷ All these features are fully described and figured in detail in Sahni (1930); see especially Text-fig. 4 and Pl. 50, figs. 6-10; Pl. 51, figs. 15-25.

these rachises agree generally with the primary petiole of *Austroclepsis*; but they vary in diameter from as little as 4 or 5 mm. to as much as 27 mm. If we ignore certain differences which are probably due to the exigencies of preservation¹, the rachises of different sizes, described under several distinct names (*C. composita* Ung., *C. robusta* Ung., *C. antiqua* Ung., *C. antiqua* var. *exigua* P. Bertrand), readily fall under one species as rachises of different orders in a single diffusely branched frond (see Fig. 1). As I have already suggested elsewhere², a plant bearing fronds of such a kind must have differed considerably from any known member of the Zygopterideae. The mere presence of a clepsydroid bundle in the leaf need not imply a generic identity of Unger's plant with the Australian zygopterid any more than with the Siberian. These points will be further elaborated in a special part at the end of the paper. The comparison with *Tempskya* refers only to the habit and, of course, implies no affinity with the Cretaceous genus. For the present the *Tempskya*-like false stem may be included as a diagnostic character of the genus; it is for the future to show whether it runs through the whole genus or has only a specific value.

The only known species of the new genus is

Austroclepsis australis (E. M. Osborn) Sahni comb. nov.

1915. Preliminary observations on an Australian *Zygopteris* E. M. Osborn (1915), p. 727 (no figures).

1917. *Ankyropteris australis* E. M. Osborn in litt., see Sahni (1919), p. 82.

1919. An Australian specimen of *Clepsydropsis*. Sahni (1919).

1928. *Clepsydropsis australis* (E. M. Osborn MS.). Sahni (1928).

SPECULATIONS ON THE HABIT AND AFFINITIES OF

CLEPSYDROPSIS UNGER

The resemblance of *Austroclepsis* with *Clepsydropsis* Unger, so strikingly shown in the foliar bundle, seems to me to express at most a distant affinity. For reasons presently to be stated, I believe that the habit and mode of branching of Unger's leaves was very different from that of both the Siberian and the Australian zygopterids.

In my paper on *Asterochlaenopsis* I hinted at the possibility "that Unger's *Clepsydropsis* rachises, with their varying diameters, represented branches of a large *Hierogramma* frond, in which case a

¹ See, on this point, the critical observations of Solms-Laubach (1890), pp. 25-7, and of Bertrand (1911), p. 4.

² Sahni (1930), p. 466.

Thuringian *Cladoxylon* would still be the parent stem of *Clepsydropsis*"¹.

Soon after that paper was sent to the press I made another European tour and was able to examine some of the other scattered remnants of the Unger Collection, preserved at Breslau, Lille and London, and to re-examine the material at Berlin. The most I can say is that my suspicions on the *Clepsydropsis*-*Cladoxylon* question were strengthened, especially by an examination of some sections in Prof. Bertrand's laboratory at Lille. If this suspected connection

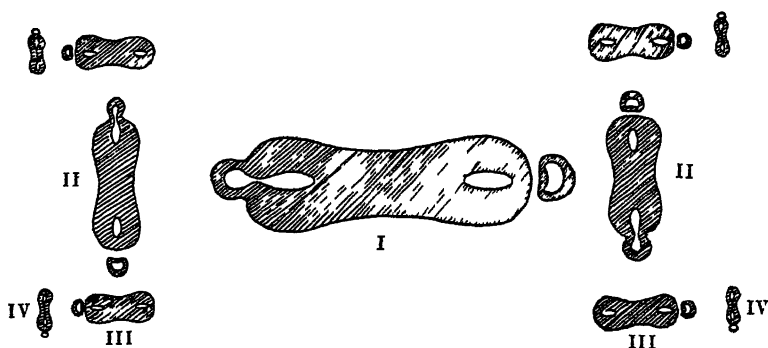


Fig 1 *Clepsydropsis antiqua* Unger Ground plan of foliar bundles showing probable mode of branching of the frond, as visualised by Paul Bertrand in his hypothetical form, *Eoclepsydropsis*. The large and small clepsyroid bundles, here shown as belonging to different orders of branching in a single frond, correspond to those of rachises previously described under the names *C. robusta* Ung (I), *C. antiqua* Ung (II, III), and *C. exigua* P Bertrand (IV). If, as suggested in the text, these "species" or "varieties" are only different conditions of *C. antiqua* Ung the fronds of this plant must have differed widely in habit from those of any zygopterid at present known.

could be established beyond doubt we should have the strongest possible grounds for removing the Australian species from Unger's genus. For the present, however, it must be admitted that this proof is lacking, and until we know better *Clepsydropsis antiqua*² must be considered entirely by itself. But even then the great variation in the diameters of these rachises³, and the little that we know of their

¹ Sahn (1930), p. 466 footnote

² Including *C. robusta* Ung, *C. composita* Ung and *C. exigua* P B, which are probably only different conditions of *C. antiqua*. See Solms-Laubach (1896), pp. 25-7, Bertrand (1911), p. 4, Sahn (1919), p. 82.

³ The diameter varies from as little as 4 or 5 mm to at least 15 mm, the structure remaining the same. In *C. robusta* Stenzel records a diameter of as much as 27 mm.

mode of branching, suggests a habit distinct from that of *Asterochlaenopsis* and *Austroclepsis*. The Saalfeld rachises seem to have belonged to a large diffusely branched frond with ramifications in two different planes, one perpendicular to the other (see Fig. 1) rather than to a leaf in which a dominant main rachis gave off minute appendages on the two sides. In such a frond, each branch rachis must have been supplied at its base by a ring-like strand which higher up became clepsydroid by tangential flattening and median constriction. This is clearly suggested by the known facts that (a) lateral bundles were nipped off as rings from the ends of the peripheral loops, and (b) at least in one case (the *C. exigua* of Bertrand) the inner ends of the two peripheral loops have been found connected together by a persisting bridge of small tracheids similar to those lining the loops themselves¹. Now if we try to visualise the habit of such a frond it will be found that the branches were in two rectangular planes, for each annular strand, as it came off, became flattened in a plane at right angles to that of the parent bundle. If from our knowledge of the behaviour of the strands we were to reconstruct the frond in a ground plan, the reconstruction would agree in a remarkable way with Prof. Bertrand's hypothetical form *Eoclepsydropsis*², which he regarded as the *Urform* of the *Zygopterideae*.

The unusual habit which this mode of branching would impart to the frond is easy to imagine. Thus, quite apart from whether *Clepsydropsis* Unger was the foliage of *Cladoxylon* or not, the available facts seem to support the idea that it was a diffusely branched frond of very peculiar habit, unlike that of any of the other plants which have been referred to *Clepsydropsis*.

It is considerations such as those set forth above that make me hesitate in regarding the Thuringian rachises, without further evidence, as co-generic with any other known plant.

SUMMARY AND CONCLUSIONS

1. Reasons are given for the view that the southern zygopterid generally known as *Clepsydropsis australis* is not a *Clepsydropsis*, in spite of its petiolar structure, but represents a new and distinct genus. For this the name *Austroclepsis* has been proposed.

2. *Austroclepsis* is distinguished from other *Zygopterideae* by a

¹ Bertrand (1911), Pl. I, figs. 1-5; Pl. II, fig. 16; (1911 a), p. 21, fig. 21; see Sahni (1919), p. 84.

² Bertrand (1909), p. 258, Text-fig. 36.

combination of *Clepsydropsis*-like, *Ankyropteris*-like and *Tempskya*-like characters. The petiolar bundle is of the *Clepsydropsis* type, but the stem stele and leaf-trace sequence agree with the corresponding organs in *Ankyropteris Grayi* or *A. scandens*, except that the leaf-trace has no adnate axillary strand. The repeatedly forked leaf-bearing axes are bound together by adventitious roots into a *Tempskya*-like false stem. The only known species is *A. australis* (E. M. Osb.) Sahni comb.nov. With the possible exception of a slender *Botrychioxylon*-like axis epiphytic among its roots, *Austroclepsis* is the only genus of Zygopterideae yet recorded from the southern hemisphere.

3. There is some evidence that in their habit and mode of branching the Thuringian leaves referred to *Clepsydropsis* differed considerably from those of both the Siberian and the Australian zygopterids and, indeed, from any zygopterids at present known.

4. Even if *Clepsydropsis* Unger was a zygopterid (which on present evidence seems uncertain) it should not be accepted without hesitation as co-generic with any other member of that family.

5. To avoid confusion it is desirable that the name *Clepsydropsis* be used in the sense of a form genus, that is, it should be reserved for rachises of unknown attribution containing vascular bundles of that type. The presence of clepsydroid strands in the leaves is not by itself a proof that the plants in which they are found belong to the same *natural* genus.

ACKNOWLEDGMENTS

I wish to express my sincerest thanks to Dr Crookall (London), Prof. Soergel (Breslau), Prof. Gothan (Berlin) and Prof. Paul Bertrand (Lille) for the courteous and liberal manner in which they gave me access to the material. To Prof. Gothan I am further indebted for permission to cut fresh sections from some of Unger's blocks, and for many other kindnesses during my visits to Berlin. Prof. Bertrand laid me under a deep obligation by sacrificing much of his time in detailed discussions, both personally and in correspondence, over the possible attribution of *Clepsydropsis* to *Cladoxylon*. It has also been my privilege, for which I am deeply grateful, to discuss this and other theoretical points with Prof. A. C. Seward, F.R.S., and Dr D. H. Scott, F.R.S.

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A NOTE ON THE ORIGIN OF LATERAL ROOTS AND THE STRUCTURE OF THE ROOT-APEX OF *LYGINOPTERIS OLDHAMIA*

By A. C. HALKET

(With Plate XI and 1 figure in the text)

LYGINOPTERIS OLDHAMIA, a plant of the Carboniferous period extremely common in the coal-balls of Lancashire and Yorkshire, was stated by Dr D. H. Scott in 1924 to be "now almost the best known of fossil plants" (5), p. 121). The general habit of the plant, the anatomy of its vegetative parts and the structure of its reproductive organs are all well known. In spite of the comparative completeness of our knowledge, however, there still exist many lacunae in the story of the life-history of this plant, some of which may be filled as new material becomes available for study. For example very little, if anything, is known at present about the development and method of growth of the various plant organs. It is advisable therefore to record some information which has been obtained recently concerning the development of the lateral roots of this plant.

The roots of *Lyginopteris oldhamia*, first described by Williamson (9) under the name of *Kaloxylon Hookeri*, are readily identified by the structure of the cortex, with its two sharply contrasted zones and secretory sacs.

Recently, while examining a number of preparations made from calcareous nodules from the British Coal Measures, some sections of well-preserved roots of *Lyginopteris* were found which show features not previously described. Two sections are of special interest. These are both of young tetrarch roots preserved before the commencement of secondary thickening, and are both cut transversely and in the plane of origin of a very young lateral root. For convenience of reference these sections will be called Root 1 and Root 2.

Root 1 occurs in slide 17, Root 2 in slide 24 in the Bedford College Collection; both preparations were cut from nodules from the Upper Foot Mine, Shore, Littleborough, and supplied by the Lomax Palaeobotanical Laboratories of the Lancashire and Cheshire Coal Research Association.

Information has been obtained from the examination of these sections relating to (i) the structure of the inner cortex, (ii) the place of origin of the lateral roots, and (iii) the structure of the root-apex.

(i) *The structure of the inner cortex.* The tissue of the inner cortex in many of the roots found in nodules from the British Coal Measures is not very well preserved and is described as "consisting of rather large cells, loosely packed, so as to leave considerable intercellular spaces between them," and as "evidently a somewhat lacunar tissue" (Scott (4), pp. 52 and 54).

The tissue of the inner cortex in Roots 1 and 2, however, is unusually well preserved and does not altogether agree with this description.

In Root 1 (Text-fig. 1 B) the tissue of the inner cortex is seen to consist of delicate thin-walled cells closely packed together so that the intercellular spaces are quite small. In parts of Root 2 the same structure can be seen, while in other parts of the tissue it can be seen that large spaces are formed by the breaking down of these cells.

The formation of these large spaces may have taken place naturally as the roots grew older, but it seems more probable, from the appearance of the tissue, that the breaking down was due to decay before fossilisation and that the young living roots did not have a definitely lacunar inner cortex such as is characteristic of marsh plants of the present day.

The characteristic secretory cells occur among the thin-walled cells and can be recognised by the darkness of their walls.

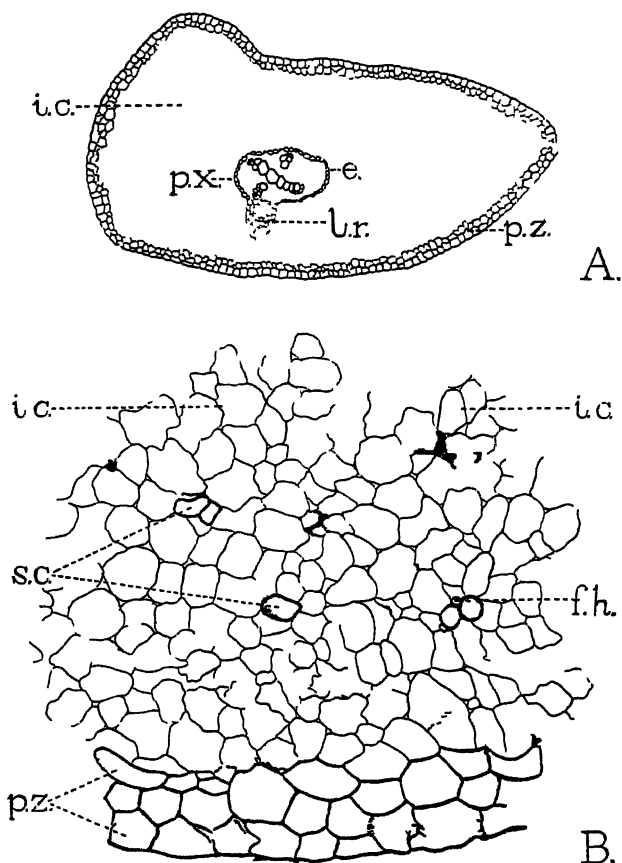
Fungal hyphae are present in the inner cortical tissue of both these roots (Williamson and Scott (10), p. 737).

(ii) *The place of origin of the lateral roots.* The place of origin of the lateral roots can be determined, since, in both sections, the developing branch root is so young that it has only penetrated about half-way through the inner cortex.

In both roots the xylem and phloem groups can be easily distinguished and it can be seen that a single layer of cells (the pericycle) separates them from a layer of closely fitting cells (the endodermis). Hence the position of each developing root in relation to the tissues of its parent root can be seen. It is clear that a lateral root was formed by the division of the cells of the one-layered pericycle and ruptured the endodermis before making its way through the cortex.

The branch roots of *Lyginopteris*, therefore, arose in the same tissue as do those of modern gymnosperms (Strasburger (7), Reinke (3)). The roots of *Lyginopteris*, however, appear from the evidence of these

sections to arise laterally to a protoxylem group not exactly opposite to it as is the case with the roots of modern gymnosperms. It should



Text-fig. 1. A, diagram of transverse section of Root 1. $\times 25$. B, part of the cortex of this root. $\times 145$. *p.z.* "peripheral zone" of cortex; *i.c.* thin-walled parenchymatous cells of inner cortex; *e.* endodermis; *p.x.* group of tracheides of protoxylem; *l.r.* very young lateral root, cut tangentially; *s.c.* "secretory cells"; *f.h.* fungal hypha.

Both A and B were drawn with the aid of a projection apparatus. The thickness of the line in B indicates the depth of colour of the cell wall, not its thickness.

be noted though that Williamson and Scott (10), p. 740) state that in older roots, with secondary thickening, the xylem of a branch root is found opposite to the protoxylem tracheids of the main root.

(iii) *The structure of the root-apex.* We have at present little information concerning the structure of the root-apex of *Lyginopteris*. In 1908 Stopes and Watson(6) figured a longitudinal section of an isolated root-apex, which, with other similar apices, was ascribed to *Lyginopteris* by Weiss in 1913(8). Weiss could come to no conclusion as to the method of growth of the root, though he was inclined to think that an apical cell may have been present as in ferns.

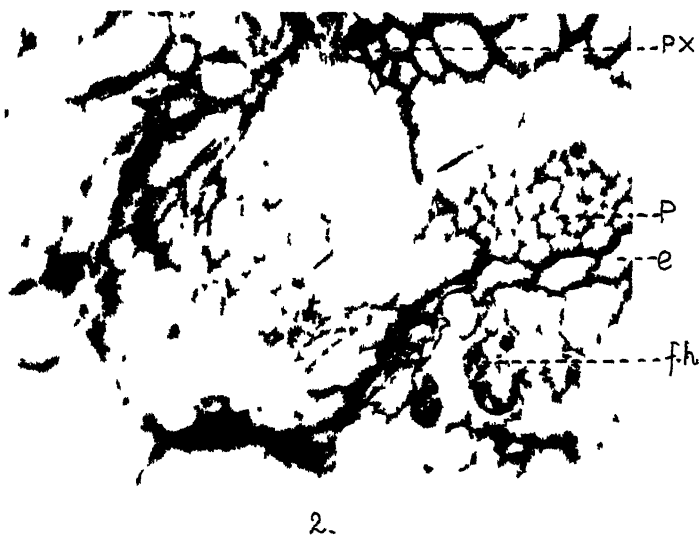
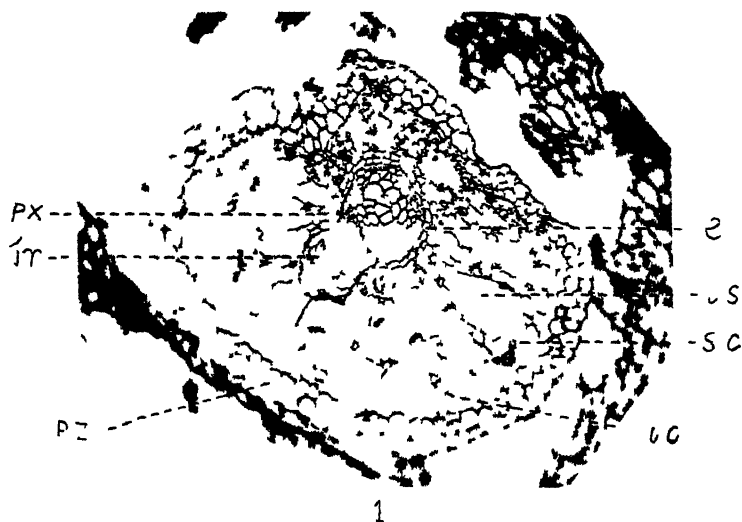
The structure of the apex of developing rootlets of this plant can be seen in Roots 1 and 2. Details of the structure can be seen better in Root 2 for the plane of the section cuts the emerging root longitudinally in the median plane, or approximately in this plane. In Root 1, on the other hand, the developing rootlet is cut tangentially.

The root-apices of fossil plants are so rarely found that it is thought advisable to record the structure of a single apex even though the conclusions drawn cannot be confirmed by comparison with other apices.

The delicate walls of the cells composing the apex of the rootlet in Root 2 are sufficiently well preserved to enable the type of organisation to be determined. The arrangement of the cells is shown in the photographs reproduced in Pl. XI. Two regions can be distinguished, a central one (the plerome) composed of some slightly elongated cells, and a larger peripheral one (the periblem) consisting of fairly regularly arranged rows of cells continued over the top of the central region. The apical part of the periblem will obviously form the root-cap. This arrangement of cells is the same as that seen in the young branch roots of gymnosperms at an early stage when the differentiation of the plerome is just beginning, but when the characteristic form of the periblem can be clearly distinguished (Strasburger(7), Tafel XXIV, fig. 28, and Reinke(3), Fig. 5). On the other hand, no sign of the presence of the large apical cell characteristic of the roots of most ferns can be seen or of the segments cut off from such a cell; neither can a group of initial cells be seen such as is characteristic of the Marattiaceous type of root.

From the arrangement of the cells in this apex, therefore, it may be deduced that the structure of the root-apex and, thence, the mode of growth of the root of *Lyginopteris oldhamia* were similar to those of modern gymnosperms, as well as to those of *Amyelon radicans*, the root of *Cordaites*, another palaeozoic gymnosperm (Halket(1))¹.

¹ References to other papers on the structure of the root-apices of gymnosperms are given in this paper.



HALKET—ROOT STRUCTURE OF *LYGINOPTERIS*
OLDHAMIA

Lyginopteris oldhamia has fern-like foliage and is one of the many plants of the carboniferous epoch which were at one time thought to be ferns. Furthermore, *Lyginopteris oldhamia* was the first of these fern-like plants proved to have borne seeds (Oliver and Scott (2)). Hence a special interest is attached to the fact that, even in comparatively minor characters of organisation, such as the structure of the apex and the vertical orientation of the diarch xylem plate in lateral rootlets (Weiss (8)), there should be agreement with modern gymnosperms rather than with ferns.

BEDFORD COLLEGE, LONDON

November, 1931

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EXPLANATION OF PLATE XI

Figs. 1 and 2 (photographs)

Fig. 1. Transverse section of Root 2. *p.z.* "peripheral zone" of cortex; *i.c.* thin-walled parenchymatous cells of inner cortex; *i.s.* large intercellular space formed by the breaking down of the cells; *e.* endodermis; *p.x.* group of tracheids of protoxylem; *l.r.* very young lateral root; *s.c.* "secretory cells." $\times 42.5$ circa.

Fig. 2. Part of same section enlarged to show the details of the arrangement of the cells as seen in the longitudinal section of the young lateral root. *p.* phloem; *f.h.* fungal hypha—other lettering as in fig. 1. $\times 212.5$ circa.

A NOTE ON THE OCCURRENCE OF ABNORMAL FLOWERS OF *NASTURTIIUM OFFICINALE* R.Br.

By A. C. HALKET

(With 1 figure in the text)

IN a recent number of this journal Dr Agnes Arber⁽¹⁾ gave an account of some abnormal flowers found in certain members of the Cruciferae. Among these were included flowers of *Nasturtium officinale* R.Br. which had accessory flowers developed from a parent flower.

Dr Arber first found these abnormal flowers in the year 1929. They grew in three localities near Cambridge; in Littondale and Wharfedale in Yorkshire; and at Bibury in Gloucestershire. In 1930, and again in 1931, similar anomalous flowers were found in one of the localities near Cambridge. The abnormality was therefore widespread in 1929, and in one of the localities at least was repeated in three successive years.

Since considerable importance is attached by Dr Arber to the recurrent nature of the abnormality, and, since the only reference to similar abnormal flowers of *Nasturtium officinale* found in the literature was a note by Irmisch⁽²⁾ in 1861, any additional information concerning these interesting flowers seems of value. It is proposed therefore to put on record the fact that similar flowers were found by the writer in Essex about seventeen years ago and to publish some notes made then.

In the early part of the summer a plant of *Nasturtium officinale*, growing in one of the drainage ditches of Pitsea marsh, low-lying land south of Pitsea station not far from the River Thames, was noticed because of the unusual appearance of its inflorescence. On examination it was found that the conspicuousness of the inflorescence was due to the fact that the flowers contained little additional flowers so that they had the appearance of "double" flowers.

These abnormal flowers were only noticed in one raceme, but may have occurred in others since the surrounding plants were not examined carefully.

The inflorescence in which they were found was young and its upper flowers were still tiny buds. All the open flowers in the basal part of the inflorescence possessed the little accessory flowers, but no examination was made to determine whether those still in the bud stage were abnormal or not. It is, therefore, not possible to say whether this plant was similar to Dr Arber's specimens in the dis-

tribution of the anomalous flowers. The abnormality in her specimens occurred "principally in the first formed flowers of main raceme" (1), p. 189).

Details of the structure of one of these flowers can be given, since notes were taken in the case of a single representative flower. This

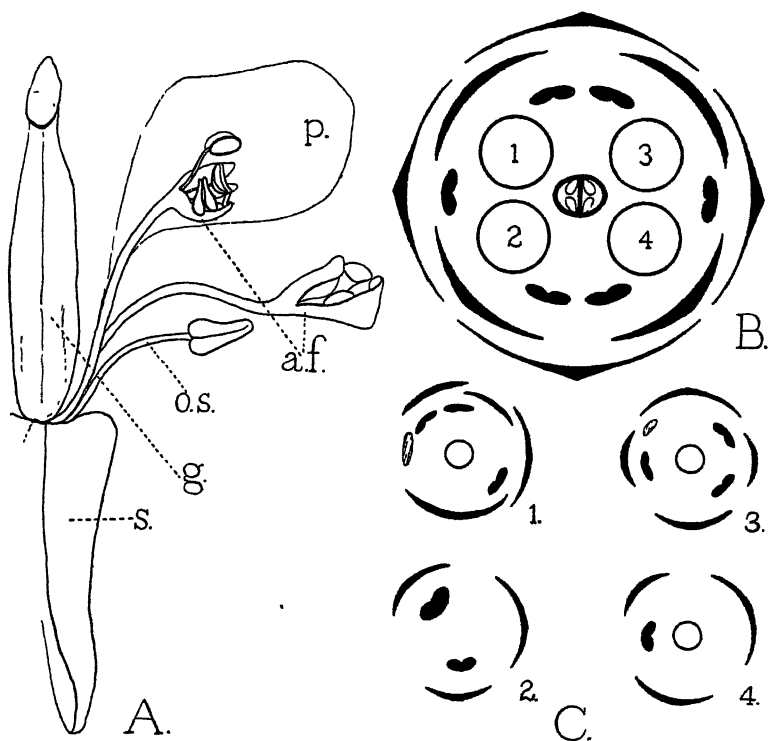


Fig. 1. A. Drawing of part of abnormal flower (very much enlarged) to show the relative positions of outer stamen, two accessory flowers and gynaecium. *g.* gynaecium; *a.f.* accessory flower; *o.s.* outer stamen; *p.* petal; *s.* sepal which has been turned back to expose the other parts of the flower.

B. Diagram of abnormal flower showing the position of the four accessory flowers, which are represented by numbered circles.

C. Diagrams of the four accessory flowers, numbered as in B. Rod-like structures (possibly staminoids) shaded.

flower was normal in all respects except for the possession of the four little additional flowers.

The small accessory flowers were all open and some of their anthers had dehisced. Nothing therefore can be said about their position in the bud or during development. In the mature flower the flowerets were not symmetrically placed, as was the case with Dr Arber's specimens, but occurred in pairs. One pair of flowerets

stood on each side of the median plane of the flower between a short, outer stamen and the ovary. Particular attention was paid to this point and a drawing made to show the position of a pair of accessory flowers relative to an outer stamen and the pistil of the parent flower. This drawing is reproduced in Fig. 1 A, and in Fig. 1 B is given a diagram of the abnormal flower in which the positions of the flowerets are shown by circles, numbered for convenience of reference.

The accessory flowers were unlike the normal Cruciferous flower and were all different from each other. Floral diagrams showing the number and positions of the parts of the four flowerets are given in Fig. 1 C, 1-4.

The perianth segments were white like petals, but, except two in floweret 3, were veined with green. The stamens varied in number in the different flowers; the anthers contained pollen except that of the single stamen of floweret 4. One of the two stamens of floweret 2 had an exceptionally large extrorse anther, and this flower had no gynaecium.

No anomalous structures were noted with the exception of small, green rod-like bodies in two of the flowerets, 1 and 3, which may have been staminoids. No unusual features of the gynaecia were noted such as might be caused by the attached pollen sacs found by Dr Arber in sections of the young buds of abnormal flowers.

No anatomical investigation of these abnormal flowers of *Nasturtium officinale* was made, so nothing can be added concerning the relation of the vascular strands of the various parts of the flower to each other.

These abnormal flowers of *Nasturtium officinale*, therefore, were similar to those described by Dr Arber, but appear to have differed in certain minor points; the accessory flowers were in pairs and were more normal in the structure of their parts; also they were approximately in the same stage of development as the parent flower, they were not in anthesis when the parent flowers were maturing fruits as appears to have been the case in some at least of Dr Arber's specimens (Arber, Fig. 9 A, 1 and 2).

BEDFORD COLLEGE, LONDON

November, 1931

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REVIEWS

ENGLER and PRANTL. *Natürliche Pflanzenfamilien*. Ed. 2, Band XIX a.

The advantage of having had for upwards of half a century an illustrated *Genera Plantarum* in their mother tongue has been the happy lot of German students of Systematic Botany. If English-speaking students had been provided with a similar work, this branch of botany might have attracted a far greater proportion of research workers than has been the case. As it is, little has been done in this country since the time of Bentham and Hooker's *Genera Plantarum*, which is quite literally a closed book to the average student impatient of the Latin text, and of the absence of illustrations. The handsome volume under review represents part of a second edition of Engler's monumental work, the corresponding part of the first edition having appeared in 1897. It deals with two of the Englerian Series, the Geraniales and Pandales, the latter by Dr J. Mildbraed, containing one family (Pandaceae), one genus (*Panda*) and one species (*P. oleosa* Pierre), a tropical African tree. We may gain some idea of the wealth of information about this tree made available to the German reader. After a long detailed description of the genus we find the passage of which I give a free translation: "The tree is distributed in the rain forest area of West and Equatorial Africa from the Ivory Coast to the Lower and Eastern Congo; in the South Cameroons it is called in the Pangwe language 'afán.' It has numerous native names in the Ivory Coast, according to Chevalier, and in the Belgian Congo, according to De Wildeman. The natives obtain from the seed an edible oil which, however, on account of the very unfavourable proportion of the seed to the stone kernel could never attain enough importance for export. . . . The wood has a specific gravity of about 0.65, is firm and tough and turned into axe-handles and is used in joinery."

The series Geraniales is the work of the late Prof. Engler. He commences with three pages of historical matter relating to the treatment of the group, much of which is now of little interest and might have been omitted. There follows an enumeration of six sub-series, most of which I believe are not really related to Geraniaceae at all. The half of the first sub-series is natural enough, Oxalidaceae, Geraniaceae, Tropaeolaceae, Linaceae, Zygophyllaceae forming a group clearly derived from a common stock. But I should not admit into it Erythroxylaceae, as Engler has done in his system. It spoils the homogeneity. And where are the Balsams, Balsaminaceae? They are so familiar in this position in Bentham and Hooker, and surely they have a close relationship with Geraniaceae and Tropaeolaceae especially? Engler places Balsaminaceae with Sapindales, i. e. alongside the horse chestnuts; they agree with the latter in having the raphe in a similar position relative to the axis, but that is probably all the resemblance there is.

There must be some significance allowed to habit in determining relationships. If not, then why are all Cruciferae herbaceous, all Malvaceae fibrous, nearly all Rubiaceae woody, etc.? I cannot believe that Cneoraceae, Rutaceae, Simarubaceae, Burseraceae, Meliaceae and Akaniaceae, nearly all unfamiliar to British botanists, but amongst which I have worked for many years, are at all related to Geraniales proper. The Meliaceae contain the Mahoganies, our hardest timbers, and the Mahoganies are not at all obviously related to the Geraniums, to put the argument in its crudest form.

We may pass over the second sub-series Malpighiineae, which I prefer to derive from the Tiliales, and consider the third and fifth sub-series, Polygalineae and Tricoccae. Both are familiar to British botanists in Polygalaceae and Euphorbiaceae respectively. The most primitive Polygalaceae are trees and shrubs like *Carpolobia* and *Securidaca*, and it is very difficult to derive trees from herbs such as Geraniaceae mainly are, whilst one can find little in common

between that family and Polygala, a very advanced genus, its herbaceous species the most advanced of all.

It is probable that Engler knew little of the detailed structure of the Euphorbiaceae. If he had he would not perhaps have so readily associated them as a sub-series of Geraniales. It was my good fortune in earlier days to obtain a working knowledge of this family by classifying the African species, and anyone who has done that cannot fail to realise that the family has been derived from several sources, such as Malvaceae, Sterculiaceae, Tiliaceae, Celastraceae, Flacourtiaceae, and Sapindaceae, but not I think from Geraniaceae.

Although some of us may criticise the phylogenetic arrangement of the Englerian System, the great value of the work as an up-to-date Genera Plantarum cannot be over-estimated. Most of the text for this part of the revised Pflanzenfamilien was done by Prof. Engler, and since his lamented death has been seen through the press by Prof. Harms. It is the most valuable modern work on taxonomic botany and should be on the desk of every working systematic botanist.

J. H.

The Carboniferous Coal District of Brandov in the Rudohori Mountains (Erzegebirge), Bohemia. By F. NĚMEJC. Tride XI. Palaeontographica Bohemiae, No. 14.

This paper makes an interesting contribution to the palaeobotanical "species problem." It is primarily a description of an ordinary coal measure flora: the commonest fossils are "impressions" of *Sigillaria* bark. Several authors have pointed out that it is hard to identify the species of *Sigillaria*; the difficulty results mainly from the great variety of bark sculpture in a single *Sigillaria* tree (comparable perhaps with the range met with in a modern fir tree) and from the considerable number of these variable species. Determination is desirable for comparison; it would be most disagreeable to leave these common fossils undescribed, and names are also needed because certain types of *Sigillaria* are rather characteristic of the rocks of certain definite ages.

The usual method of specific determination is to arrange the specimens in groups which may contain specimens differing rather widely provided the extremes seem to be connected by intermediates. This series is then matched with a similar series by someone else; if only a few specimens are available, one has to be content with only a few intermediates. This gives an author great scope for expressing his "individuality."

Němejč's method is quite different. He thinks it hopeless (at present) to try to disentangle natural species out of this welter of bark types. Instead he matches individual specimens and places them in a good many "species" and "sub-species" or "forms," which he recognises as almost entirely artificial. As a standard, he uses the monograph of Koehne, and to simplify matters he uses this as his only standard. He describes the specimens under such names as *S. aff. fossorum* forma *Ebertiana*, and in each case selects one of Koehne's specimens as "typical" of each form. This is of course much easier and less subjective than putting specimens into series and matching the whole series.

Many palaeobotanists will not share Němejč's view that true species of *Sigillaria* are unrecognisable; his method, however, does not impede progress towards the recognition of these true species, for it is possible to discuss which of these forms belongs to a single species quite as well after describing them under names as before; indeed Němejč does this at some length although without any simple results. His determinations can, of course, be converted into those of an ordinary "splitter" by leaving out the word "aff."

His real contribution is in giving up the concept of a "species" which had been arrived at for the study of recent plants, and using instead another concept and methods which seem to him better suited to this class of fossil material. Though the "artificial species" is less aesthetically attractive than the "natural species" if it simplifies and so aids work it must be regarded as an advance.

T. M. H.

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NOTES ON NEW AND LITTLE-KNOWN ALGAE FROM THE BEDS OF RIVERS

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(With Plate XII and 7 figures in the text)

INTRODUCTION

ON stones and other submerged objects on the bed of almost any shallow stream there can be found a large number of algae. A brief description of such growths studied by means of submerged glass slides was given by Butcher, Pentelow and Woodley⁽¹⁾ in an account of the investigations on the River Lark. Though the growths on the slides in the spring consist of well-known diatoms, the summer growths comprise for the most part rare and little-known algae—chiefly members of the Chaetophoraceae and Chamaesiphonaceae, and it is amongst these that a number of rare or hitherto unknown species have been encountered. Similar growths have already been described by Geitler⁽²⁾ and Fritsch⁽³⁾, and to the accounts published by them the present paper adds further knowledge of these forms by the description of the following species:

Sporotetras pyriformis sp. et gen.nov.

Sphaerobotrys fluvialis sp. et gen.nov.

Ulvella frequens sp.nov.

Stigeoclonium falklandicum Kützinger⁽⁴⁾, var.nov. *anglicum*.

Stigeoclonium farctum Berthold⁽⁵⁾, var.nov. *rivulare*.

Gongrosira incrustans (Reinsch⁽¹¹⁾), Schmidle⁽¹³⁾.

Chaetopeltis megalocystis Schmidle⁽¹²⁾.

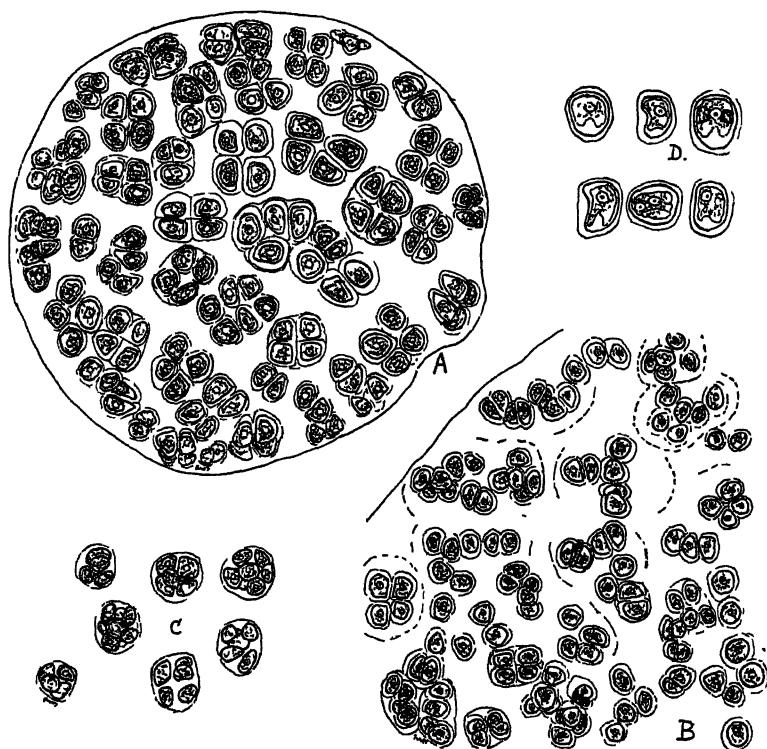
It is possible that some of the new forms are juvenile stages, but in spite of prolonged search no indication of further development has been found. Since the forms in question are among the commonest of the algae on a river bed and play an important part in the biology of the river, it is necessary to assign a name to them and so there seems to be no alternative to describing them as new.

CHLOROPHYCEAE

PALMELLACEAE

Sporotetras gen.nov.

Sporotetras pyriformis sp.nov. (Text-fig. 1; Pl. XII, A). This is the only species of the genus so far observed. The youngest stage of this alga consists of an attached sub-globose or flattened, mucilaginous



Text-fig. 1. *Sporotetras pyriformis* sp. et gen.nov. A, a small entire colony. B, portion of a much larger colony. C, groups of 4-8 celled daughter colonies. D, isolated cells showing contents and variation of shape. All from River Tees. A-C, $\times 800$; D, $\times 1000$.

mass of 4-8 cells. The cells appear to divide very rapidly and regularly in one plane by successive walls at right angles to one another, so that later the colonies are seen as rounded masses of mucilage in which the cells are grouped in fours around the periphery and within 2μ of the surface of the mucilaginous envelope (Text-fig. 1, A). The cells are

4–5 μ wide and 5–6 μ long, irregularly rounded in surface view; whilst in side view they often appear elongated or pyriform, with the narrow end directed towards the centre of the colony (Text-fig. 1, D). After recent division, which is always in a plane at right angles to the mucilage surface, the cells are flattened on contiguous sides. The daughter cells then form independent membranes of their own and, for a time, the enveloping wall of the parent cell can still be distinguished around many of the groups of four, giving the whole assemblage the appearance of lying in stratified mucilage. At this stage the colony is fixed to the substratum by about one-sixth of its surface and in the region of attachment the mucilage is wrinkled, so that the shape of the plant can best be likened to that of certain fungi such as *Scleroderma vulgare* and *Phallus impudicus* (in the very young stage), that is, spherical except in the region of attachment. Internally the colony is devoid of cells and either hollow or occupied by structureless mucilage. It has not been possible to demonstrate the presence of pseudo-cilia. The chloroplast is a lobed parietal plate situated towards the outer side of the cell, and it contains a single large and distinct pyrenoid (Text-fig. 1, D).

In the oldest colonies observed the outline of the mucilage is irregular and not so convex; the cells are considerably smaller (3–4 μ diam.), more rounded and not so distinctly pyriform in side view, nor so regularly aggregated in groups of four (Text-fig. 1, B). At this stage independent masses of mucilage containing a variable number of cells have been observed (Text-fig. 1, C) in and about the main mass; these appear to represent parts detached from it and most probably develop into daughter colonies.

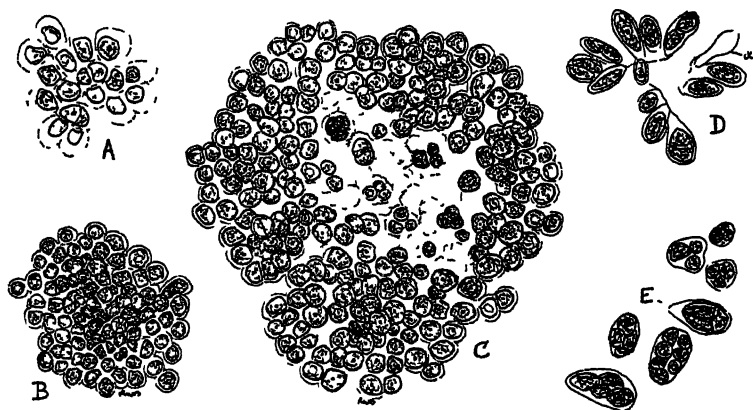
This plant, though obviously related to *Tetraspora*, differs chiefly in the variable and frequently pyriform cells, the apparent absence of pseudo-cilia, the character and position of the chloroplast, and the close proximity of the cells to the mucilage surface.

This alga is not uncommon in the lower reaches of the river Tees from June to September. It has also been observed in the Hull.

Sphaerobotrys gen.nov.

Sphaerobotrys fluviatilis sp.nov. (Text-fig. 2; Pl. XII, C, D). This is the only species so far observed. In its youngest stages it consists of a flat colony of 4–16 sub-globose cells. The latter are rounded or polygonal through mutual pressure, and each cell is surrounded by a wide but ill-defined mucilage envelope (Text-fig. 2, A). The cells divide by walls at right angles to the substratum, so that a

mature plant shows the cells densely grouped, after the manner of a bunch of grapes, into a cushion-shaped or hemispherical colony with an approximately circular outline (Text-fig. 2, *B*). There is no evidence in these colonies of more than a single layer of cells and this botryoidal form appears to be attained by the mutual pressure of the quickly dividing component cells which are displaced upwards. At this stage of the development of the plant the mucilaginous envelope is very thin around the outer cells, but in the centre, especially in that part devoid of cells, it is often thick and brown. The cells are now circular in surface view ($3-4\ \mu$ diam.), but are elongated or pyriform ($3-5\ \mu$ long) in side view, arranged with the narrow end towards the



Text-fig. 2 *Sphaerobotrys fluviatilis* sp. et gen. nov. *A*, young plant of 16 cells. *B*, a small mature plant. *C*, an older plant with groups of cells in the centre. *D*, portion of plant pressed out to show the elongated cells and empty sheath (v). *E*, cells showing division of contents pressed out from a mature plant. *A-C* and *E* from River Tees, *D*, from River Hull. $\times 800$.

centre of the colony. Further, the most markedly pyriform cells show a prolongation of the mucilage towards the centre (Text-fig. 2, *D*) and often also, division walls parallel to the substratum. The chloroplast apparently occupies the whole of the peripheral cytoplasm of the cell and contains a single ill-defined pyrenoid.

In the oldest plants observed many of the central cells have lost their contents and show an empty sheath, pyriform in side view, whilst the remainder of the cells exhibit division of contents by successive divisions into four or eight cells (Text-fig. 2, *C* and *E*). These presumably are the reproductive bodies, and, so far as can be ascertained, they are formed by the protoplast dividing into a number of

parts which acquire new membranes of their own before escaping. At other times reproduction seems to take place by the liberation of individual cells or groups of cells without preliminary division. Specimens of this alga do not reach any considerable size, they are usually 0.5–0.7 mm. diam., and the largest seen was 1.5 mm. diam.

This alga seems to be common and widely distributed, as it has been collected in the Rivers Lark, Tees, Itchen, Cam, Hull, but never in great quantity.

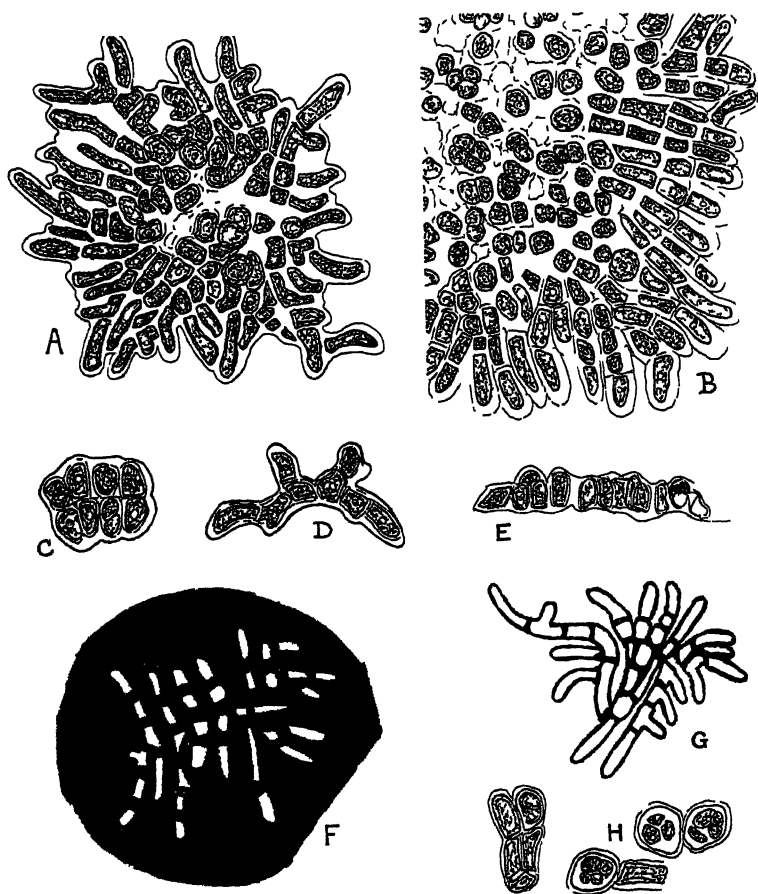
CHAETOPHORACEAE

Ulvella frequens sp.nov. (Text-fig. 3; Pl. XII, *B* and *C*). The youngest stage of this alga consists of an unbranched or branched irregular filament (Text-fig. 3, *D*) or an aggregate of 4–8 isodiametric or elongate-polygonal cells (Text-fig. 3, *C*). Later, a small one-layered disc, with an entire or irregular outline is formed (Text-fig. 3, *A* and *B* and Pl. XII, *B* and *C*), in which the central cells are more or less angular and but little elongated, while the peripheral cells are elongated into short radiating filaments. These peripheral filaments are frequently branched and the branches are usually formed just beneath the terminal wall of the cell, and it is not until the outgrowth has reached a length equal to that of a normal cell that it is cut off by a septum. The whole plant is surrounded by a well-developed and often thick envelope of mucilage which stains very easily with methylene blue and other dyes. In still older plants the peripheral cells are often not so markedly elongated, and the cells in the centre of the disc lose their compact arrangement and become irregularly grouped, while rounded cells are cut off by walls parallel to the substratum and to the surface of the disc. These central cells then exhibit division of the protoplast to form apparent reproductive cells. From the few examples seen it seems that these apparent reproductive cells result from a simultaneous tetrahedral division of the contents of the upper cells (Text-fig. 3, *H*). Zoospores have not been observed, but the cells in these probable sporangia remain naked.

Each cell possesses a single parietal chloroplast, usually with a single pyrenoid, though often this is very indistinct. The walls are thick and gelatinous, without evident stratification. The average size of a central cell is 4–5 μ diam., of a peripheral cell 4–5 \times 8–12 μ .

There is a considerable range of variation in several of the characters. The mucilage may form a broad, apparently circular investment around the whole plant, it may form a thin envelope around each individual cell, or may exhibit all grades of development between the two extremes (see Text-fig. 3, *F* and *G*). The outline of the discs may

be circular, elongated or lobed, and the relative development of the peripheral filaments and of the central parenchymatous portion also varies. There are also differences in the length and the extent of development of the peripheral cells in different plants. Usually the



Text-fig. 3. *Ulvella frequens* sp. nov. *A*, a small entire plant. *B*, portion of much larger plant. *C* and *D*, two forms of very young plant. *E*, vertical section. *F*, plant with broad mucilaginous envelope. *G*, plant with narrow mucilaginous envelope. *H*, cells pressed out from mature plants showing division of contents. All from River Tees. $\times 800$.

plant forms discs between 0.5 and 1 mm. The largest disc (about 3 mm. diam.), as yet positively referred to this species, is shown in Pl. XII, *B*, but it is not out of the question that still larger discs may be found.

Ulvella frequens is an abundant alga during the summer in all rivers so far examined, viz. the Itchen, Lark, Cam, Tern, Ure, Tees and Hull. It seems never to develop large growths, so that it is easily overlooked amid the thick crusts formed on the stones by other algae.

One other fresh-water species has been recorded in this country by Beesley⁽¹⁾ from springs near Fleam Dyke, Cambridgeshire, and this has been named *U. Beesleyi* by Fritsch in West and Fritsch⁽¹⁴⁾. The two organisms are closely related, but differences are found in the development of the central portion and in the arrangements of the peripheral cells. In the mature thallus of *U. Beesleyi* the central portion consists of a dense aggregation of parenchymatous cells with little sign of disintegration of the cell contents in older plants and there is a greater contrast between the central and the peripheral portions. Production of biciliate zoospores has also been recorded in this species.

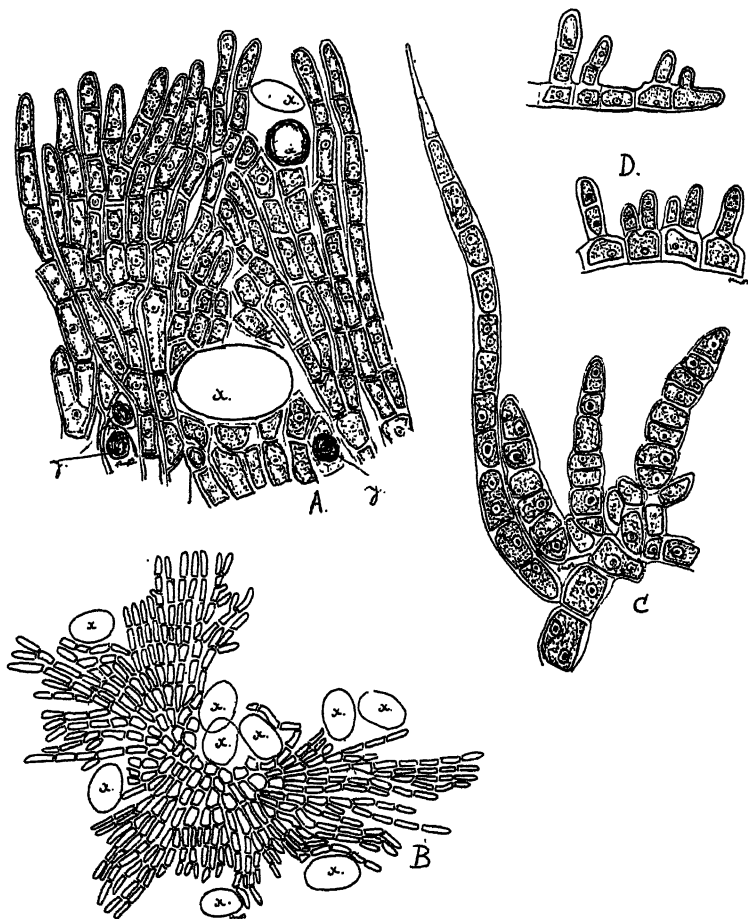
U. frequens also shows a certain superficial likeness to *Protoderma viride* Kütz., especially to the plants figured by Borzi⁽³⁾. His drawings of normal young plants of *Protoderma* show the same type of branching and the same differentiation into the central and peripheral regions. In older plants there are marked differences. In *P. viride* the thallus of the central portion remains compactly arranged as a single layer of pseudo-parenchymatous cells, but in *U. frequens* the central portion of the thallus soon loses the compact character seen in the younger discs.

Stigeoclonium

The prostrate portions of various species of *Stigeoclonium* are often abundant on submerged slides and are extremely variable. Occasionally well-developed erect branches may be observed growing out from the prostrate portion, but in most cases only the latter is at all well developed. In most of the recognised species of *Stigeoclonium* the prostrate system is unknown or inadequately described, and it therefore seems advisable to describe in detail what has been observed on the submerged slides. The reference of these forms to existing species must, however, be considered tentative.

Stigeoclonium farctum Berthold⁽²⁾ nov. var. *rivulare* (Text-fig. 4; Pl. XII, F, G) ["*Nov. Gen. Ulvacearum* Reinsch⁽¹¹⁾" (Taf. IV)]. Prostrate system of a rounded and almost entire outline, very variable, but always well developed and formed of filaments which are closely adjacent throughout their length. The most characteristic plants are discs of almost circular outline, up to 2 mm. in diam. (Pl. XII, F) and

formed by filaments of almost equal length which radiate from a few regularly arranged, central polygonal cells. These filaments are sparsely branched and the branches usually arise on both sides of the main



Text-fig. 4. *Stigeoclonium farctum* Berthold nov.var. *rivulare*. A, portion of prostrate system showing the periphery, method of growth round obstacles (x), and erect branches (y). B, a complete young plant to show general method of growth and branching. C, young erect branches. D, vertical section of edge of plant. All from River Tees. A, C and D, $\times 800$; B, $\times 250$.

axis. They grow out from beneath the septum and form a very acute angle with the main branch, frequently lying almost alongside it. They may be alternate, or opposite and are of about the same width

as the main branch, so that the latter appears to branch dichotomously. These laterals may branch repeatedly in the same manner, and in this way the various filaments occupy the whole of the available area covered by the disc. Frequently foreign objects, such as diatoms, may be seen within the thallus with the branches growing around them. In other plants the disc is oval or irregularly lobed, but there is the same close and regular arrangement of the filaments. The outer cells of these prostrate systems are narrowly oblong ($8-10 \times 3-4 \mu$) and the inner cells oblong to square ($4-5 \times 4 \mu$), there being very little difference between the width of the cells in primary or secondary filaments. The chloroplast is a parietal plate, sometimes folded over at the edge and contains one or two usually conspicuous pyrenoids.

The erect system (Text-fig. 6, C), which is usually poorly developed in comparison to the prostrate system, grows simultaneously from most of the central cells of the disc. The apex of an erect filament is at first blunt, but later acute, acuminate or sometimes terminated by a hair. The filaments are very delicate and narrow (4μ in diam.), sparsely branched, with short and alternate branches. The cells are very variable in length, being either isodiametric or elongated, with contents identical to those of the prostrate system. Hairs are very rarely produced and have been more frequently seen in samples left growing under laboratory conditions than in plants taken direct from the river.

One of the characteristics of this plant is the large development of the prostrate system and the paucity of the erect system. Exceptional specimens may show a considerable development of the latter, and in these cases the filaments are scarcely distinguishable from those of *St. falklandicum* described below.

This plant is provisionally referred to the *St. farctum* of Berthold (?), but it shows a more considerable range of development, especially in the erect system. The distinguishing features of Berthold's species are the strong differentiation of the prostrate system, and the weak development of the erect system which consists in the main of hairs. Hairs are very rare or absent in the many forms observed by me, while the erect system may at times be well developed. The general character of the prostrate system and of its cells seems to agree with Berthold's description and, since the species of *Stigeoclonium* are known to be exceedingly variable, it seems advisable to include the forms above described under *St. farctum*. Owing to the usual lack of hairs in the natural habitat and the occasional abundant

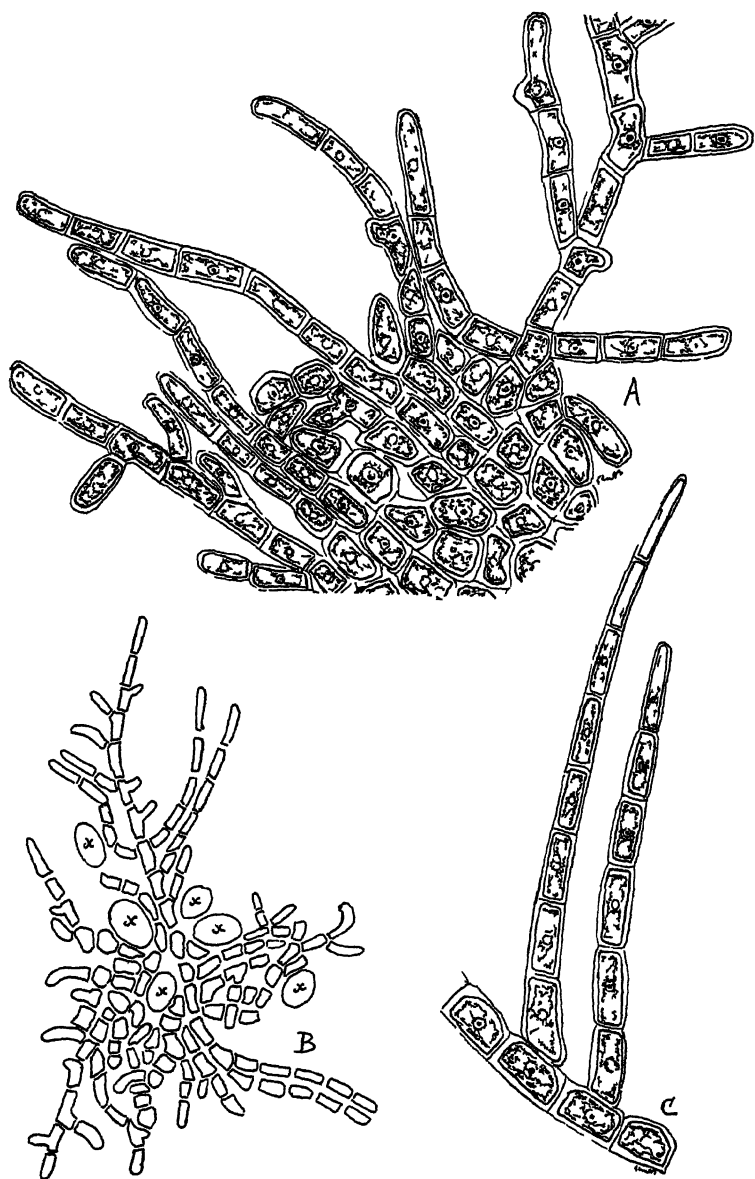
development of the erect system, however, it would appear that we are dealing with a distinct variety which may be referred to as *St. farctum* var. *rivulare*.

This alga is generally distributed in the Tees, Lark, Itchen and Hull, being usually most abundant in calcareous waters.

Stigeoclonium falklandicum Kützing (9) nov. var. *anglicum* (Text-fig. 5, Pl. XII, E). The prostrate system is exceedingly variable. In some cases it consists of a single filament of subglobose cells, either branched or unbranched; or at the other extreme, it forms discs with an entire outline, approaching those of *St. farctum* var. *rivulare*. In typical plants the prostrate system is a lobed disc which has an irregular outline owing to the unequal length of the component filaments and their branches. These filaments may be almost simple or irregularly branched, and the branches can arise from any part of the longitudinal wall of the cell and from either side of the main filament. As in *St. farctum* the branch commences as a protrusion of the cell of the main filament and the septum is formed later. The branches are very irregular and divergent and stand off at angles between 90° and 10° from the main filament. The inner cells are also rather larger ($5-7\ \mu$), while the outer are rather wider ($6-7\ \mu$) than in *St. farctum*. In all cases the disc of *St. falklandicum* can be distinguished from that of *St. farctum* by the larger, more nearly isodiametric central cells, by the branches being very irregular, divergent and not closely apposed to each other, and by the irregularity of the margin caused by the unequal growth of the component filaments. The chloroplast is a parietal plate in which are one or more, usually indistinct pyrenoids.

The erect filaments develop simultaneously, but only from a few of the central cells of the disc, and arise early in the life of the plant, so that most individuals have both disc and erect portion well represented. The general form of the erect filaments is almost identical with that found in the exceptionally well-developed specimens of *St. farctum*, but there are sometimes slight differences. Thus the filaments seem stouter ($7-8\ \mu$ instead of $5\ \mu$), the pyrenoid is not so distinct, and the proportion of erect to prostrate system is considerably greater. There is the same paucity of branching in the young plants and the same variation in type of apex which may be acute, acuminate or setaceous. The best developed plants so far observed show considerably greater branching in the upper portion, and this is of the same type as is found in *St. tenue*.

St. falklandicum was described and figured by Kützing from the



Text-fig 5 *Sigeoclonum falklandicum* Kutzing nov var *anglicum* A, portion of prostrate system showing the periphery B outline of a complete young plant to show general method of growth and branching (x) obstacles formed by *Cocconeis* C, young erect branches All from River Tees A and C, $\times 800$, B, $\times 250$.

Falkland Islands (10). Both description and drawings are very meagre, but there is a general resemblance between his and my plants that seems to justify their inclusion under one name. Since, however, there are some differences, the plant above described is best referred to as a distinct variety, namely *St. falklandicum* var. *anglicum*.

This plant is generally distributed, though not common, throughout the River Tees and doubtless occurs elsewhere.

Stigeoclonium tenue Kützing (9) (Text-fig. 6, A). This is a commoner and better known species, especially so far as the erect portion is concerned. It has frequently been observed on the submerged slides in the Tees, together with *St. farctum* and *St. falklandicum*, but is always readily distinguishable by the stoutness of the main axis of the erect filaments, the abundance of branches and the poor development of the prostrate system. This last usually consists of 5–20 sub-globose cells arranged in the form of an irregular filament, from which usually only one erect branch arises, as figured by Huber (8). The figure here given (Text-fig. 6, A) will serve for comparison with the two species previously discussed, and the form in question seems to correspond exactly with Kützing's drawings (10) of *St. tenue* var. *gracile* which has erect filaments with apices considerably more acuminate than in the type.

It is obvious that there is a close relationship between the three plants just described. In the first place, as has been pointed out by many other observers, the extent of development of the prostrate and erect systems seems to be complementary. In *St. farctum* the prostrate system is large and the erect system small, in *St. tenue* the reverse is the case, while *St. falklandicum* occupies an intermediate position. Although the similarity of the erect systems of *St. farctum* and *St. falklandicum* may suggest that they are varieties of the same species, the time of development of the erect system is earlier and its extent compared to the prostrate system considerably greater in the latter than in the former. The prostrate systems moreover seem to be very different and, though they frequently occur side by side on the same slide, they develop in a totally different manner (see Text-figs. 4, B and 5, B). The main difference seems to rest in the mode of branching. In *St. farctum* the branches arise from just beneath the septum, while in *St. falklandicum* they originate from the lateral walls, some way beneath the septum, but these different modes of branching may be related to the rapidity of growth and the closeness with which the filaments are aggregated. Even in respect of branching, however, there are several individuals which are difficult to assign to either species.



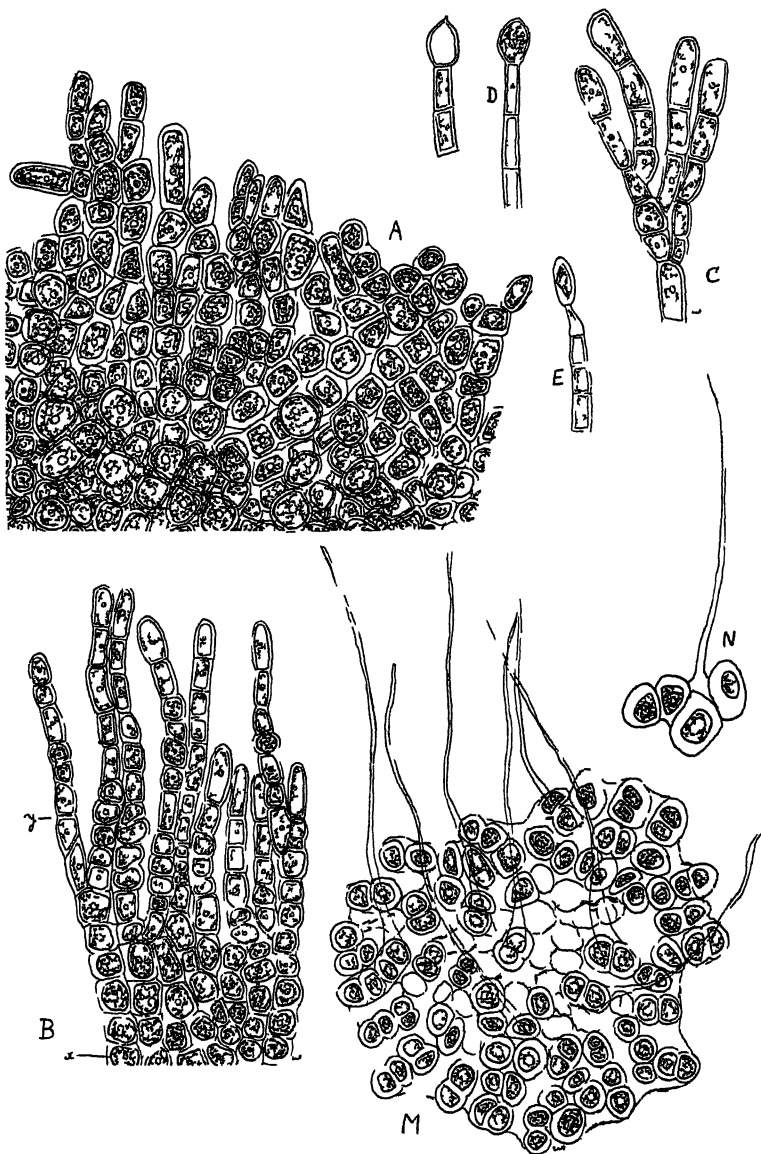
Text-fig. 6. Erect portion of *Stigeoclonium* spp. A, *St. tenue*. B, *St. falklandicum* var. *angustum*. C, *St. farctum* var. *rivularis*. The whole of the prostrate system is shown in A and only portions in B and C. All from River Tees. $\times 300$.

The method here employed for studying these sessile algae yields growths consisting chiefly of the prostrate system which has mostly been ignored by other workers. On the other hand the erect system rarely develops to any extent, so that the difference in the more frequently observed prostrate systems warrant the two forms being kept distinct.

TRENTEPOHLIACEAE

Gongrosira incrustans Schmidle(13) [*Chlorotylum incrustans* Reinsch(11)] (Text-fig. 7, A-E; Pl. XII, H). The youngest stages of this plant are very variable and usually consist of a few isodiametric or polygonal cells. Sometimes these are grouped to form a few irregular filaments arising from a central pseudo-parenchymatous mass, and sometimes only the latter is formed. In the former case there is superficial resemblance to the prostrate system of certain species of *Stigeoclonium* (Pl. XII, H), and in the latter case a resemblance to the youngest stages of *U. frequens* may be seen. Although the cells are enveloped in mucilage, this does not stain strongly with methylene blue, and thus the young stages of the alga can at once be distinguished from those of *U. frequens*. The young plants soon become encrusted with a calcareous deposit, and the next stages show the central cells divided to form a many layered, pseudo-parenchymatous, cushion-shaped mass in which the elongated cells of the peripheral region of the basal portion are barely distinguishable. The general form of the plant as seen from the upper surface at this stage, after the calcium carbonate has been dissolved, is shown in Text-fig. 7, A. Each pseudo-parenchymatous cell is $4-5\mu$ in diameter and contains a chloroplast in the form of a parietal plate, mostly granular and containing a single, usually distinct, pyrenoid.

The mature plants appear on stones and on the glass slides as hard minute roundish bodies, about 0.5 mm. in diameter and suggestive of the minute perithecia of certain lichens. At this stage a vertical section shows a base of polygonal cells (Text-fig. 7, B) forming a pseudo-parenchyma 2-6 layers deep in which the cells are arranged in more or less evident upright series, no doubt corresponding to joined threads, and passing over apically into dense masses of sparsely branched filaments each 5μ in average width. These free filaments are irregular and made up of cells of various shapes varying from sub-globular ($6 \times 6\mu$) to cylindrical ($5 \times 10\mu$). Branches appear to arise in two different ways; some of the uppermost cells terminating the rows of the pseudo-parenchyma have apparently branched dichotomously although probably at first laterally (Text-fig. 7, B), while



Text fig 7 A-E *Gongrosira incrustans* Schmidle A portion of young plant. B, vertical section of older plant showing pseudo-parenchyma (v) and erect filaments (1) C, an older branched erect filament D and E filaments producing akinetes. M, N *Chaetopeltis megalocystis* Schmidle M, mature plant N, young plant with a single bristle All from River Tees $\times 800$

later in the development of the upper portion the filaments branch from almost every cell (Text-fig. 7, C). These latter branches arise from the longitudinal wall just beneath a septum and show eversion (Text-fig. 7, C). Unlike *Stigeoclonium* and *Ulvelia* the young branch appears to be cut off by a septum from the main axis almost immediately. The cells of these erect filaments like those of the parenchyma contain a parietal chloroplast and a single pyrenoid.

The apical cell of a growing filament is usually cylindrical and obtuse. Several filaments have, however, been isolated in which the apical cells are ovoid or irregular and these are illustrated in Text-fig. 7, D. Although no zoospores, nor other reproductive cells, have actually been observed it seems reasonable to conclude that these swollen cells represent reproductive bodies which are possibly of the nature of akinetes.

Only a scanty description of *Gongrosira incrustans* was published by Reinsch (11), but so far as can be seen the vegetative portion of the plants under consideration, as well as the tendency to form lime incrustations, are markedly similar in the two. As for reproductive organs there is a difference of opinion. Reinsch described lateral protrusions from the main thread which were hyaline and had liberated zoospores. On the other hand Schmidle (13) states that the sporangia are not known. So far, I have rarely observed such protruding cells in the lower part of the filament, and if so, they always contained a chloroplast like the rest of the cells. For the present the alga above described is best referred to *G. incrustans* (Reinsch) Schmidle, and the above description adds further data on its growth and development.

G. incrustans is abundant in the lower part of the River Tees, in the Swale, Skerne and Lark, and is doubtless common in calcareous streams.

CHAETOPELTIDACEAE

Chaetopeltis megalocystis Schmidle (Text-fig. 7, M, N). This plant consists of a single layer of isodiametric cells of irregular outline embedded in mucilage to form a more or less rounded disc, while the whole plant is embedded in a fainter common envelope which is apparently less consistent. The protoplasts are widely separated from each other by the thick walls. A radial arrangement of the cells is not obvious, especially in older colonies. The bristles borne on the disc are numerous, simple and of considerable length. They are of the same width throughout except at the very base where they come away from the mucilaginous envelope, and here they are often somewhat enlarged (Text-fig. 7, N). In some individuals they

are absent. The chloroplast occupies most of the peripheral portion of the cytoplasm and contains a small indistinct pyrenoid which is surrounded by a broad starch sheath similar to that of *Ch. orbicularis* Berthold as noted by Huber (6).

My plants bear a considerable resemblance to the form described as *Ch. megalocystis* by Schmidle (12) and show very little resemblance to *Ch. orbicularis* Berthold (2) to which the former species is referred by Heering (7) as f. *megalocystis*. Neither the shape of the cells, nor the nature of their chloroplasts, nor the irregular arrangement of the cells in the mucilage suggest *Ch. orbicularis* Berthold. On the other hand there is definite resemblance to *Ch. megalocystis* in the size, shape and arrangement of the cells, in the thickness of the mucilaginous cell walls and in the apparently entire chloroplast. The main point of difference is that in my form the walls are much thicker, giving the whole disc a rather different aspect, but on this point alone it is scarcely justifiable to separate it from Schmidle's species.

This alga is common and generally distributed in the upper Tees throughout the summer, but has not so far been observed in any other river.

DIAGNOSES OF NEW SPECIES

The following are brief Latin diagnoses of new genera and species described above:

Sporotetras gen.nov.

Cellulae irregulariter rotundatae saepe elongatae plerumque pyriformes, 4 vel 8 laxe circum marginem corporis mucilaginosi dispositae. Pseudo-cilia absunt. Chromatophora cyathiformis pyrenoide singulo in parte latiore cellulae. Propagatio per fractionem coloniae matricalis in partibus parvis globosis mucosis cum cellulis VI, tetraedricis dispositis vel pluribus fit.

Sporotetras pyriformis sp.nov.

Plantae juvenales e cellulis in corpore globoso gelatinoso tenaci quaterne dispositis constantes. Cellulae, 10 μ diametro, irregulariter rotundatae vel pyriformes post divisionem in superficie proxima deplanatae. Chromatophora cyathiformis pyrenoide singulo in parte latiore cellulae. In familiis senioribus corpus gelatinosum irregularis et cellulae minores subglobosae haud evidenter pyriformes vel distincte dispositae.

Sphaerobotrys gen.nov.

Cellulae rotundatae vel pyriformes in corpore mucoso subsphaerico vel irregulariter aggregatae. Cellulae periphericae interdum e tegumento gelatinoso generali parum prominentes. Chromatophora parietalis in parte peripherica totae cellulae disposita pyrenoide singulo magno, saepe indistincto. Propagatio per divisionem cellularum in parte media familiae fit.

Sphaerobotrys fluviatilis sp.nov.

Plantae juvenales deplanatae, e cellulis subglobosis IV–XVI in ambitu vaginae gelatinosae saepe tenuis dispositis. Plantae seniores pulvinatae vel hemisphaericae cellulis rotundatis vel pyriformibus dense racemiformatae aggregatis, in parte marginali divergentibus. In statu adolescenti cellulis medianis maxima ex parte resolventibus, reliquae propagatio fiunt. Chromatophora parietalis in parte peripherica cellulae disposita.

Ulvella frequens sp.nov.

Plantae juvenales e filo irregulari brevi vel disco deplanato cellulis paucis irregularibus constantes; cellulae medianae disci isodiametricae externis elongatis. Tota planta in vagina gelatinosa inclusa. Plantae seniores e disco rotundato vel ovali vel irregulari constantes cellulis internis elongatis parallelibus vel divergentibus; cellulis internis interdum parum rotundatis. Postremo cellulae medianae plures disintegrant et reliquae in planitie horizontale divisunt; contentes cellularum supernarum in corporibus reproductivis verisimiliter sese divisunt.

GENERAL REMARKS ON THE SPECIES

The species just described were found under circumstances that were distinctive in two respects. They were obtained from growths on glass slides submerged for a month in the running water of the stream. The substratum of glass is in some respects artificial, although not greatly different from the flint stones that are frequent in the Lark and Itchen. Were these algae found on glass alone or were the same forms always present no matter where the glass slides had been submerged, there would be grounds for suspecting that the substratum was an important factor in determining the forms that develop thereon. These growths have, however, proved to differ considerably

at different times of the year, and also at the same time in different parts of a stream's course and in different streams.

Recognisable fragments of many of the forms described, including comparatively large plants such as *Stigeoclonium farctum* and *Ulvella*, have been collected on threads of *Cladophora* and *Oedogonium*, while others have been observed on the stones of the stream bed. It is obvious, however, that it is very difficult to pare off from a stone in a recognisable form, the complete one-layered prostrate system of a *Stigeoclonium* or the very small discs of *Ulvella*, and this fact alone will account for the juvenile forms here described being rarely recorded hitherto.

The status of the algae described in this paper remains doubtful. Whether they are mature forms, or in part juvenile stages, only further investigations will show. The rate of growth is very variable and evidently depends on a variety of factors. Although all slides were left submerged for a month, in some months a very thick growth would appear, while in others there was very little growth indeed.

The other environmental factor to be considered is current. All the forms described have water moving continually in one direction over their surface, but there are not sufficient data to indicate what is the actual effect of this current in determining the form and distribution of these algae.

The new and little-known forms described here are among the commonest growing on the glass slides that have been submerged at various times since 1926 in the Rivers Tees, Lark, Itchen, Cam, Tern and Hull. Several other better known algae that are also frequent in these rivers are:

Coleochaete scutata Bréb. A few examples occur in the summer throughout the River Tees.

Chamaesiphonopsis regularis Fritsch. This is one of the dominant algae in all the above rivers during the months of June, July and August.

Chamaesiphon incrustans Grunow. This is not uncommonly associated with *Chamaesiphonopsis regularis* and is widely distributed, though rare.

Chamaesiphon curvatus Nordst. has been found from time to time in the Tees, but not commonly.

Oncobyrsa cesatiana Rabenhorst has been found rarely in the Cam, Lark and Tees.

Phormidium foveolarum Gomont. This is very common and widely distributed among the thicker growths of such species as *Gongrosira*

incrustans and *Stigeoclonium* spp., and has also been found in almost pure thin growths on the glass slides.

Phaeodermatium rivulare Pascher has been found rarely in the Lark and Tees.

Heterolagynion Oedogonii Pascher is a very abundant organism in the Lark, Cam, Itchen and Tees during the periods of summer floods.

In addition a large variety of diatoms occur and these are particularly abundant in the spring months.

It is also worth noting that, compared to Fritsch's lists from Devon(5) and Geitler's lists from Austria(6a), Schizophyceae and Chrysophyceae are extremely rare in the rivers I have examined. I have not met with *Hydrurus foetidus* recorded by West(14) from Devon, Yorkshire and Scotland and apparently common in the mountain streams of the continent. *Oncobyrsa* spp. are very rare and *Xenococcus* has not been observed.

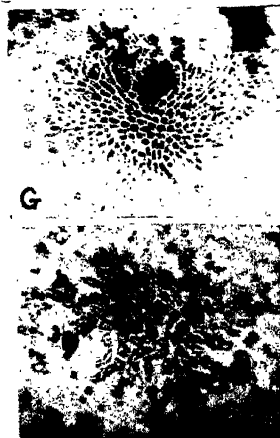
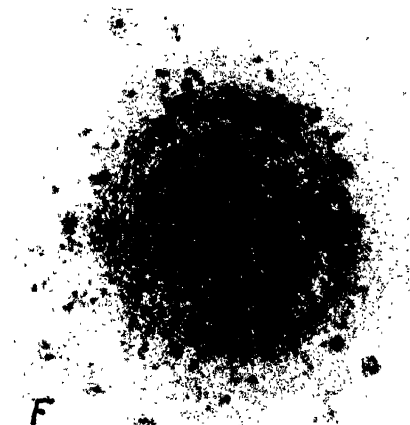
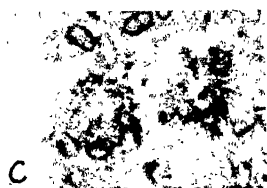
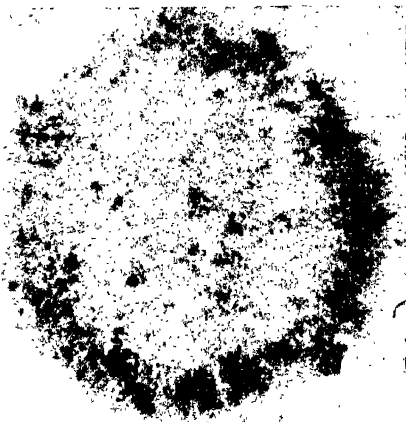
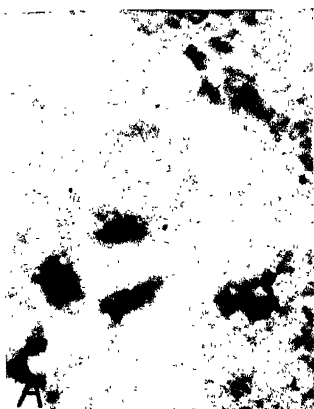
ACKNOWLEDGMENTS

A great deal of the work was carried out in the Botany Department of East London College, University of London, and the author wishes to express his thanks to the college authorities for facilities afforded him, but especially to Dr F. E. Fritsch, Professor of Botany, for his valuable advice upon these difficult forms.

The plants from the River Tees were obtained in the course of a comprehensive survey of the river, now being undertaken for the Department of Scientific and Industrial Research, as part of the programme of the Water Pollution Research Board, and the author wishes to express his thanks to the Department for permission to publish details in this paper in advance of the full report which is in preparation. The plants from the River Hull were obtained by Messrs N. C. Akers and F. W. Oliver, and the author also tenders his best thanks to them.

EXPLANATION OF PLATE XII

Photographs of encrusting algae as growing on submerged glass slides. *A*, *Sporotetras pyriformis*; portion of large colony. *B*, *Ulvella frequens*; a large plant. *C*, small plants of *Sphaerobotrys fluviatilis* (right) and *Ulvella frequens* (left). *D*, *Sphaerobotrys fluviatilis*. *E*, *Stigeoclonium falklandicum* var. *anglicum*. Young plant. *F*, *G*, *Stigeoclonium farctum* var. *rivulare*. *F*, well-grown plant. *G*, a young plant. *H*, *Gongrosira incrustans*, a very young plant. *A*, *B*, *F*, from River Tees. *C*, from River Itchen. *D*, *E*, *G*, from River Lark. *H*, from river Skerne. *A*, $\times 600$; *B*, $\times 200$; *C-E*, $\times 300$; *F*, $\times 100$; *H*, $\times 500$.



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MEIOSIS IN DIPLOID AND TRIPLOID *HEMEROCALLIS*

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(With 9 figures in the text)

THE object of the present study is to give an account of the structure of the paired chromosomes seen during the later stages of meiosis in *Hemerocallis* and to show how the configurations that are observed in the meiotic division of a non-hybrid triploid plant can be related to those occurring in the reduction division of an ordinary diploid one.

MATERIAL AND METHODS

The chromosome numbers of the following species and varieties of *Hemerocallis* were examined in root tip mitotic divisions and where possible in pollen mother cell divisions for meiosis. Root tips were fixed in 2BE and cut at a thickness of 20 μ . The sections were then stained by Newton's gentian-violet method. Pollen mother cells were fixed by the smear method. Medium Flemming, Benda and 2BE were employed as fixatives, gentian-violet being used for staining (vide La Cour, 1931).

LIST OF SPECIES, ETC., EXAMINED

Diploid.

Hemerocallis aurantiaca Bak. (pollen mother cells).

H. fulva L.

H. fulva \times *H. flava*.

H. flava L. (pollen mother cells).

H. Middendorffii Traut. et Mey.

H. Elmusae (pollen mother cells).

H. hipppeastrioides

H. vermusae (pollen mother cells).

Plants received under these names from the Elwes collection. Mr J. E. Dandy of the British Museum kindly informs me that he can trace no authority for these names.

Triploid.

H. fulva var. *Kwanso* Regel (pollen mother cells).

All the forms were diploid, with a chromosome number of $2n = 22$, except *H. fulva* var. *Kwanso*, which bears double flowers having no female organs and is a triploid clone with 33 chromosomes.

These numbers agree with those given by Takenaka (1929), except that his *H. fulva* was also a triploid while mine was diploid, and is evidently the type from which the triploid arose.

The somatic chromosomes throughout the species examined were of very similar types. Individual chromosome forms were not recognisable, but types could be distinguished. Each complement contains three pairs of long chromosomes with median or sub-median attachment constrictions and from two to four pairs with sub-terminal attachments (Figs. 1, 3, 4, 7 and 9).

MEIOSIS

Satisfactory prophase stages of meiosis could not be obtained in this material. Even at diakinesis the chromosomes are too condensed for observation of fine details of structure (Fig. 2).

At metaphase the chromosomes present a characteristic appearance in side view of the equatorial plate of the spindle. In the diploid species the chromosomes all pair to form 11 bivalents, while in the triploid variety *Kwanso* trivalents, bivalents and univalents occur.

The configurations seen at metaphase can all be explained by the theory that the pairing of homologous chromosomes at metaphase and the maintenance of their association until anaphase is conditioned by the formation of chiasmata (Darlington, 1931 *c*). Each chromosome is longitudinally split into two half-chromosomes or chromatids and a chiasma is formed between homologous chromosomes by an exchange of partners among these chromatids, which always associate in pairs. The shape of each configuration is determined by the number and position of the chiasmata present—in conjunction with the position of the attachment constriction on the paired chromosomes.

In the majority of organisms these chiasmata are formed more or less at random along the lengths of the paired chromosomes at the diplotene stage of meiosis. As prophase proceeds, the forces of repulsion acting between the attachment constrictions compel the chiasmata to travel towards the distal ends of the chromosomes, i.e. terminalisation occurs, often giving end-to-end association of the chromosomes by "terminal chiasmata." This may result in (a) reduction of the number of chiasmata by telescoping them at the chromosome ends to form terminal chiasmata, or (b) merely pushing

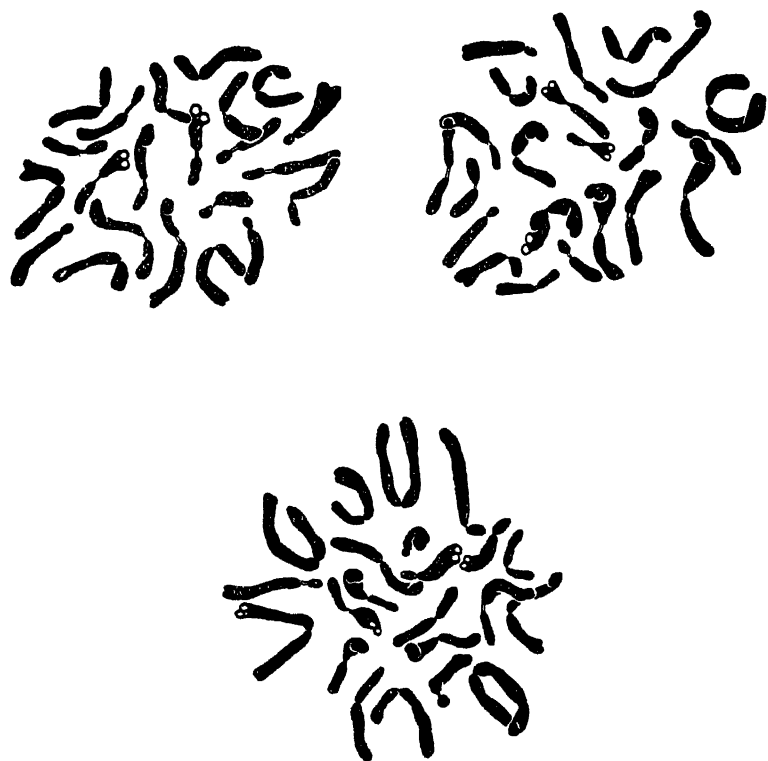


Fig. 1. Somatic chromosomes in root-tip divisions of *Hemerocallis* spp. Top left: *H. fulva*. Top right: *H. flava*. Bottom: Hybrid *H. fulva* \times *H. flava*. $\times 3750$.

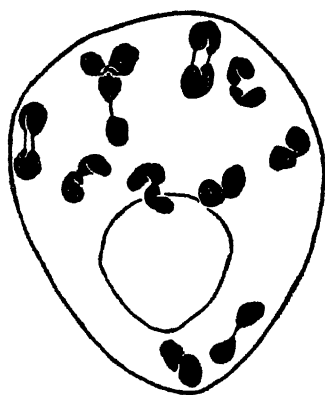


Fig. 2. Diakinesis in *H. flava*, showing the condensed form of the bivalents. $\times 3750$.

the distal interstitial chiasmata along to the ends to form terminal chiasmata, while the interstitial loops adjust their relative sizes, tending towards a state of equilibrium (cf. Darlington and Dark, 1932).

In *Hemerocallis* as in the majority of flowering plants the chromosomes at metaphase are sometimes too small and condensed to demonstrate chromatids and chiasmata directly. In these cases we can, however, infer that these structures are responsible for the observed configurations:

(i) By analogy with similar configurations found in more favourable material in which the structure can be followed in detail.

(ii) By study of the forms shown by the separating homologous chromosomes at anaphase.

A test of the correctness of this inference is that on the basis of the mechanics of chromatid and chiasma behaviour, predictions of possible configurations can be made and these are verified by the finding of the configurations during the study of meiosis in various organisms, such as in the case of *Oenothera* which was formerly believed to have association of chromosomes only end to end ("telosynapsis"), an assumption now no longer tenable (Darlington, 1931 *b*; Catcheside, 1931 *a, b*).

Fig. 3 shows a side view of a metaphase plate in which the bivalents have been separated laterally in drawing. Below is a diagram of the chromatid structure represented in the bivalents. Here it will be seen that all the chiasmata are either terminal or sub-terminal. In the division represented in Fig. 4 the first and eighth bivalents from the left-hand side have both terminal and interstitial chiasmata as shown in the chromatid diagrams below.

The thin threads in the bivalents may be supposed to be due to the forces of repulsion, already assumed to act between the attachment constrictions in terminalisation, being strong enough to stretch the chromatin material. This happens where a chiasma occurs close to the attachment constriction, which is more usual in short bivalents. The stretching continues until at anaphase the chromatids distal to the chiasma holding the bivalent together come apart and allow the chromosomes to separate as they move towards the poles. As they come apart from each other the tension is released and the chromatids snap back as if they were elastic to form small projections (see Fig. 9, rightmost pair of chromosomes). The length of these separate arms depends upon the length of the chromatids, in the original bivalent, that was distal to the chiasma, for these portions of the chromatids

are separate at anaphase while the proximal parts are closely paired and stuck together. This process is illustrated in Fig. 5, which shows successive stages in the unravelling of similar bivalents from meta-



Fig. 3. *H. vermusae*. Top: metaphase of mitosis in somatic division. Middle: side view of metaphase I in meiosis. For clearness the bivalents are spaced out laterally in drawing. Bottom: line diagram of the metaphase I bivalents showing the chromatid interpretation of their structure. The repulsion between the attachment constrictions is indicated by the arrows. $\times 3750$.

phase to telophase, and in Fig. 6 showing anaphase of a complete nucleus with chromatid diagrams of the important types.

Type A is derived from the metaphase configuration shown in Fig. 5; B comes from a configuration like No. 2, Fig. 3, but differing

from it in having a sub-terminal chiasma in the left arm (cf. No. 6); C is given by No. 7, Fig. 3; D arises from No. 1, Fig. 3; while E results from the bivalents represented in Fig. 3 as Nos. 5, 8, 10 and 11.

The size of the bead of chromatin between the two members of a bivalent is an indication of how nearly terminal is the chiasma, for the bead consists of the distal arms of a sub-terminal chiasma, hence the longer the arms the bigger is the bead.

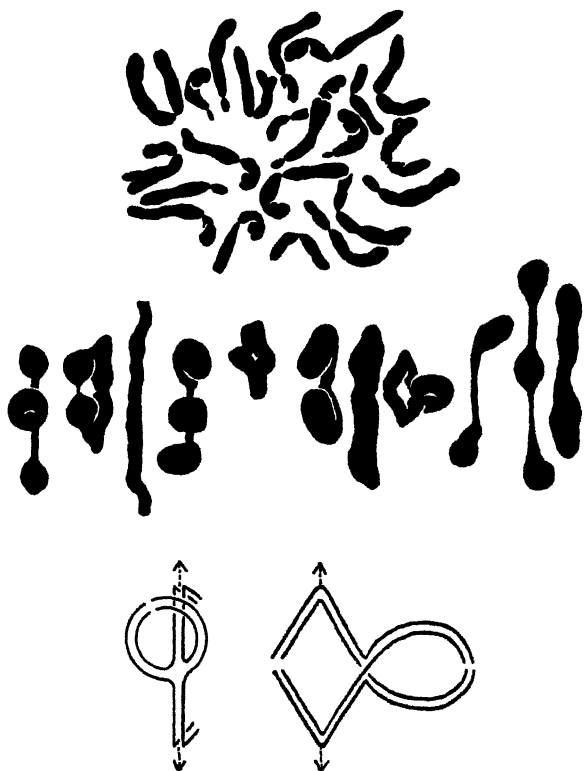


Fig. 4. *H. aurantiaca*. Top: somatic division. Middle: side view of metaphase I in meiosis. Bottom: line diagram of the chromatid structure of the first and eighth bivalents from the left in the metaphase shown above. $\times 3750$.

THE TRIPLOID *H. fulva* var. *Kwanso*

Examination of metaphase in the triploid showed univalents to be present in all cells, so that the occurrence of 11 trivalents must be a very rare phenomenon. Belling (1925) illustrates this condition in side view and Takenaka (1929) gives a not very convincing polar view

of a cell with 11 trivalents, and remarks on the rarity of complete trivalent formation. Generally there are 5 or 6 trivalents present with 6 or 5 univalents and bivalents. Only two of the four possible configurations of three chromosomes with complete terminalisation have been seen (Darlington, 1931 *a*); these are the chain formation,

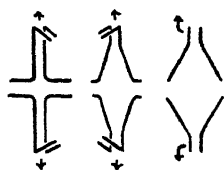
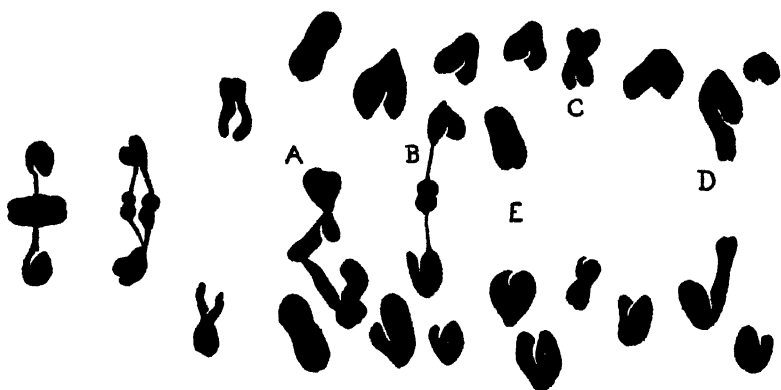


Fig. 5.

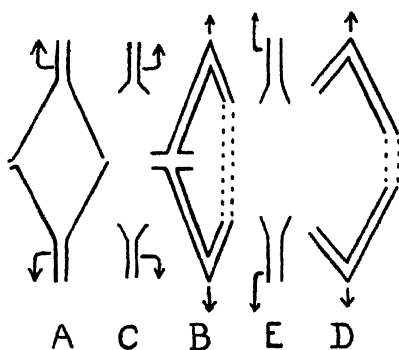


Fig. 6.

Fig. 5. *H. aurantiaca*. Three similar bivalents at metaphase I, anaphase I and telophase I of meiosis, showing stages in the unravelling of a chiasma, with line diagrams of the respective chromatid structures below. $\times 3750$.

Fig. 6. *H. aurantiaca*. Anaphase I of the meiotic division with line diagrams below of the chromatid structures of the lettered bivalents. $\times 3750$.

involving two simple terminal chiasmata (Fig. 8, 1st, 2nd, 4th and 6th trivalents) and the Y-trivalent, where all three chromosomes meet in a "triple chiasma," which is produced by terminalisation in the same direction of two interstitial chiasmata between three chromosomes (Fig. 8, 3rd and 5th trivalents). The triple chiasma type

is formed when the chromosomes have terminal or sub-terminal attachment constrictions.

The non-appearance of the more complicated trivalent configurations may be accounted for in two ways: (1) They may have been present but not distinguishable at metaphase. In the case of triploid *Primula sinensis* this was so, for although the frying-pan trivalent configuration was the commonest observed at diakinesis, only very few were seen at metaphase (Dark, 1931). (2) The chiasma frequency at diplotene may be very low, so that there is only an extremely small chance of getting more than two chiasmata between three chromosomes. Further, the chromosomes that form these configurations

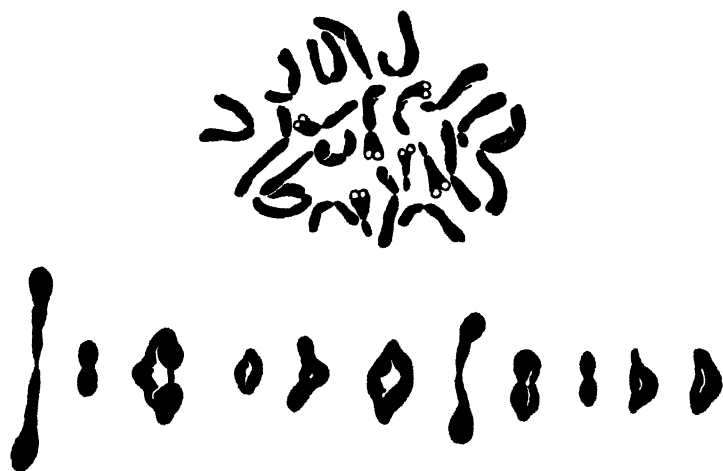


Fig. 7. *H. Middendorfii*. Top: metaphase of mitotic division. Bottom: side view of metaphase I of meiotic division. $\times 3750$.

must have nearly median attachment constrictions and in *Hemerocallis* there is apparently some peculiarity of prophase pairing by which this type often tends to form a ring bivalent and univalent instead of one trivalent.

Metaphase pairing was formerly considered to be directly due to affinity between homologous chromosomes. In a non-hybrid clone such as this¹, each individual possesses three similar sets of chromosomes. Hence, on this assumption, as pairing is complete in the

¹ The clone is considered by systematists to be a variety of the diploid species *H. fulva* which it resembles in appearance. Since no tetraploid *Hemerocallis* species are known, the triploid must have arisen by the functioning of an unreduced gamete and not by hybridisation.

diploid, the triploid should have regularly 11 trivalents—or perhaps none at all, depending on the “strength of the affinity.” The fact that a varying number of trivalents is formed could not be predicted upon this affinity assumption, but is to be expected according to the theory

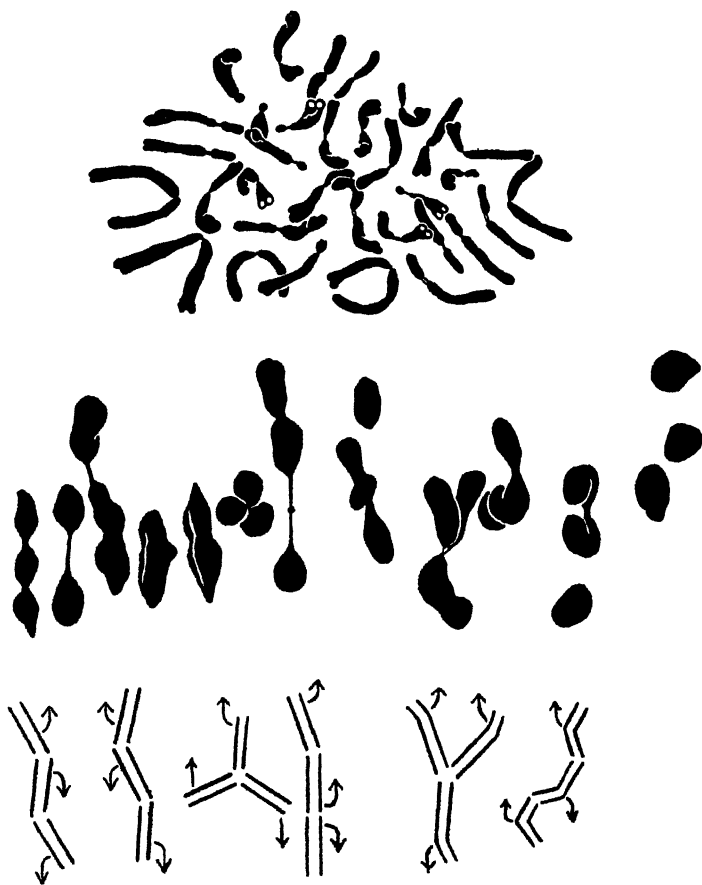


Fig. 8. *H. fulva* var. *Kwanso*. Top: metaphase of mitotic division. Middle: side view of metaphase I of meiosis. Bottom: line diagram of chromatid structure of the trivalent configurations shown in the figure above. The third and fifth from the left each have a triple chiasma. $\times 3750$.

that metaphase association is due to chiasma formation between the parts of chromosomes paired at zygotene. For in a triploid, at zygotene, only two chromosomes are associated at any one point (Darlington, 1931 c). Hence if the association between two members of a set of three homologous chromosomes was very extensive, the

chances of a chiasma forming between the free portion of one and the third chromosome would be correspondingly reduced and a bivalent and univalent would be the result (Darlington and Mather, 1932).

Micro-nuclei are formed in the pollen mother cell divisions and produce irregular "tetrads" containing various numbers of pollen grains. As Takenaka states, these arise from stray chromosomes. Since the metaphase chiasma frequency is low, one rarely meets with configurations that are lagging in their anaphase separation owing to difficulty in the unravelling of complex arrangements of chromatids, and only occasionally can lagging univalents be seen dividing on the equatorial plate after the other chromosomes have travelled to the

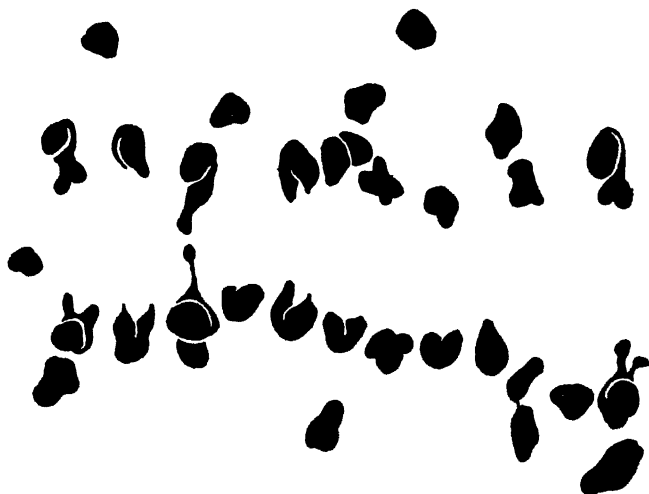


Fig. 9. *H. fulva* var. *Kwanso*. Anaphase I of meiotic division. $\times 3750$.

poles in telophase. The formation of micro-nuclei appears to be chiefly due to metaphase univalents being situated too far from the equatorial plate to be included with the other chromosomes as they separate at anaphase. Hence at interphase they form nuclei of their own.

SUMMARY AND CONCLUSIONS

The present account shows that the structures of the pairing chromosomes in *Hemerocallis* can be explained in terms of the relationship of the chromatids (longitudinally split chromosomes) at chiasmata, and their behaviour at anaphase can be interpreted on the same basis.

The failure of pairing of the third chromosome of each kind in the triploid clone (which is variable) can be regarded as due to the failure of chiasma formation (which is also variable), in the manner required by the chiasma theory of metaphase pairing. In no other way evident at present can this result be predicted.

I am deeply indebted to Dr Darlington for his help and advice throughout this study.

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EXPERIMENTS ON THE PERCEPTION OF GRAVITY BY ROOTS

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(With 3 figures in the text)

THE question of the localisation of perception of gravity in roots has long been of interest to botanists. In 1908, Haberlandt(4) gave a summary of the results published up to that date and concluded that, while both the tip and the growing region of the root are sensitive to gravity, the former is much more sensitive than the latter, and that curvature of the growing zone is caused by the conduction of excitation from the tip.

Attention has recently been redirected to the subject by a series of experiments described by Keeble, Nelson and Snow(6), which indicate that the function of the root tip in the perception of gravity may not be so simple as previous investigators have supposed. These authors, using seedlings of *Zea Mays*, found that if the tip from a root which had been geotropically stimulated were stuck on to the stump of a decapitated root which had not been so stimulated, geotropic curvature followed. They also found that if the tip from an unstimulated root were stuck on to the stump of a root, which had been decapitated and then geotropically stimulated, a geotropic curvature was again obtained. Cholodny(2) had previously performed a somewhat similar experiment but obtained negative results. Earlier experiments described by Brauner(1) show that, with oat coleoptiles too, an unstimulated tip can cause response in a stimulated stump, while Stark(10) showed that a stimulated tip can produce response in an unstimulated stump.

Keeble, Nelson and Snow account for their results by accepting the hormone theory of Cholodny(2) and Went(11), in which tropic curvature is supposed to be caused by a redistribution of a growth-regulating substance or "hormone." They suggest that "this redistribution of the growth substance can take place both in the tip and the elongating region," in which case, "the two ways in which the replaced tips affect the stumps can readily be understood. Firstly, from a geotropically stimulated tip the growth substance diffuses out in unequal concentration on the two sides, and causes an unstimulated stump to curve. Secondly, from an unstimulated tip it diffuses

out on all sides equally; but on passing into a stump which has been geotropically stimulated, it is there somehow redistributed so as to reach the upper and lower sides in different concentrations."

These results appear, at first sight, to be directly opposed to the statolith theory of geotropism, since, if such a mechanism exists, by which growth substance diffusing out equally from an unstimulated root tip can become redistributed in the stimulated stump, it is clear that perception and response can take place without any displacement of the statolith starch in the root cap. Statolith starch is not present in the growing region of the root of *Zea Mays*, and hence the geotropic sensitivity of the growing region, demonstrated by Keeble, Nelson and Snow(6), must be due to some perception mechanism other than movable starch grains. Haberlandt(4) has already shown that the growing region of the root is slightly sensitive to gravity, and it is probable that the statocyte, or cell containing statoliths, is merely the most highly evolved form of gravitational sense organ and that any cell can perceive gravity to a certain limited extent. The low percentage response (10.5 per cent.), obtained by Keeble, Nelson and Snow in their experiments with unstimulated root tips stuck on to stimulated root stumps, would seem to suggest that the perception mechanism in the stump is a very feeble one as compared with the more efficient mechanism in the tip.

It was pointed out to me by Dr T. A. Bennet-Clark that it would be of interest, firstly, to find out if the growth substance becomes unequally distributed in the tip of a root placed horizontally and, secondly, if this be the case, to find out if this power of redistribution of growth substance in the root tip is stronger than the similar power of redistribution in the stump.

Dolk(3) has already established the fact that redistribution of growth hormones takes place in the tip of stimulated oat coleoptiles, but it does not necessarily follow that this is also the case in roots. A similar experiment to that of Dolk was therefore carried out with roots of *Vicia Faba* (variety Early Long Pod) in the following manner. Seeds of *Vicia Faba* were germinated in damp sawdust and seedlings with roots between 6 and 12 cm. long were selected for experiment¹.

¹ The experimental methods described by Cholodny(2) were followed as closely as possible. The roots were allowed to dip into water for 10-15 min. before the commencement of the experiment. All experiments were carried out in a damp chamber, in the dark, at a temperature of between 10 and 15° C. The roots were sprayed with water at intervals during the experiments. By this means the roots were prevented from wilting and were always found to be quite healthy at the end of an experiment.

Two-thirds of the number used were stimulated in a horizontal position in a damp chamber for 3 hours. The remaining third were placed in the damp chamber with the roots vertical. At the end of this period, the tips of the stimulated roots were cut off and were divided longitudinally into two halves (the upper and the lower halves as shown in Fig. 1). Small plates, 2 mm. square, had previously been cut from a thin sheet of 10 per cent. gelatine. The number of gelatine blocks used was the same as the number of unstimulated roots. Four of the half-root tips which had been lowest during stimulation were placed on each of half the number of gelatine



Fig. 1. Method of preparing half tips for extraction of growth-substances. *u* = half which was uppermost during stimulation, *l* = half which was lowest during stimulation.

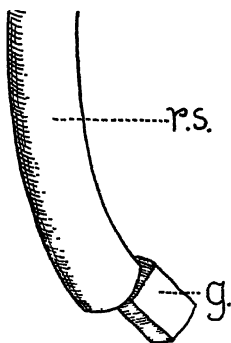


Fig. 2. Root with gelatine block attached, (after response). *r.s.* = root stump, *g* = gelatine block.

blocks. The remaining gelatine blocks each received four half-tips which had been uppermost during stimulation. The tips were then left standing on the gelatine for 1 hour in order to allow the growth hormones to diffuse into the gelatine. This method of extracting growth hormones was devised by Went (11) and has been used successfully by him and others (including Dolk in the experiment already mentioned). The roots which had been in a vertical position were then decapitated and the small blocks of gelatine (from which the root tips had been removed) were stuck eccentrically on the cut surface of the stumps, by means of a drop of warm gelatine (see Fig. 2). Thus half the roots received blocks of gelatine into which

hormones from the *lower* halves of the stimulated tips had diffused (*A*) and the other half received blocks into which hormones from the *upper* halves of the stimulated tips had diffused (*B*).

TABLE I

	Number of seedlings used	Percentage response	Average curvature
<i>A</i> (gelatine containing hormones from <i>lower</i> halves of tips)	24	91.6	30.1
<i>B</i> (gelatine containing hormones from <i>upper</i> halves of tips)	24	66.6	10.9

The results obtained are set out in Table I, from which it is quite clear that the growth-regulating substance had accumulated to a greater extent in the lower half of the stimulated root tip.

Response was in all cases *towards* the block of gelatine, thus providing further evidence for the assumption that the growth substance retards the growth of roots(7). Control experiments with pure gelatine showed that this curvature was not caused by the gelatine itself but must have been due to the hormones which had diffused into the gelatine from the root tips.

It is thus clear that when a root is stimulated by being placed in a horizontal position an accumulation of growth substances takes place in the lower half of the tip.

Accordingly a series of experiments¹ was devised in order to ascertain whether this redistribution in the tip was stronger than the hypothetical power of redistribution in the stump suggested by Keeble, Nelson and Snow(6). The experimental methods were similar to those described above and the same variety of broad bean was used. Four series of experiments were carried out as follows (about fifty seedlings being used in each series):

(a) A number of roots were decapitated and placed in a horizontal position for 3-4 hours, after which these roots were re-headed with tips from other roots which had also been stimulated in a horizontal position for the same period of time. These tips were stuck on in such a manner that the side of the tip which had been lowest during stimulation was exactly *opposite* the side of the stump which had been lowest during stimulation (Fig. 3*A*). The roots were then placed in a vertical position.

(b) A number of roots were treated similarly to those in series

¹ A preliminary account of this series of experiments has already been published (5).

(a), but the new tips were stuck on in such a manner that the side of the tip which had been lowest during stimulation was on the *same* side as the side of the stump that had been lowest during stimulation (Fig. 3 B).

(c) The roots from which the tips had been cut to re-head series (a) were also placed in a vertical position.

(d) Normal roots were stimulated and then placed in a vertical position.

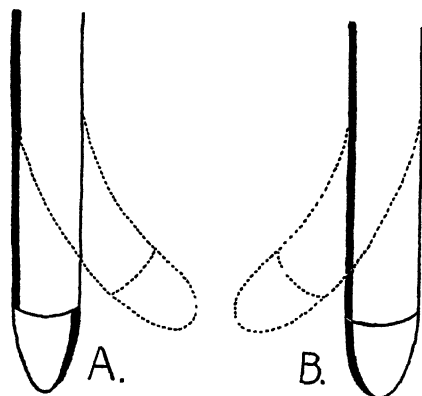


Fig. 3. *A*, diagrammatic representation of a root in experiment 2, series (a). *B*, diagrammatic representation of a root in experiment 2, series (b). The thick black line represents the side which was lowermost during stimulation and the dotted line represents the position of the root after response.

The following results were obtained 24 hours after the commencement of the experiment:

Series (a): 67.5 per cent. curved towards the side of the tip which had been lowest during stimulation.

15 per cent. curved towards the side of the tip which had been uppermost during stimulation.

17.5 per cent. remained straight.

Series (b): 70 per cent. curved towards the side which had been lowest during stimulation.

30 per cent. remained straight.

Series (c): 63 per cent. gave a positive curvature.

37 per cent. remained straight.

Series (d): 70 per cent. gave a positive curvature.

30 per cent. remained straight.

It was noted that in series (a) and (b) some of the roots which remained straight showed a swelling at the join between tip and stump which, according to Snow(9), indicates an imperfect join. Such roots were disregarded in calculating the percentage response.

In the case of those roots in series (a) which curved towards the side of the tip which had been *uppermost* during stimulation, a comparison with the actual roots in series (c) from which the tips used to re-head series (a) had been cut, showed that most of the latter had not curved, indicating that the roots from which the tips had been cut were below the average in sensitivity. Thus, when the tips used were cut from roots of low sensitivity, the power of redistribution of growth hormones in the growing region became apparent and curvature was towards the side of the stump which had been lowest during stimulation instead of towards the side of the tip which had been lowest.

Similar results were obtained with the primary root of seedlings of *Zea Mays*, but fewer seedlings were used and the percentage response was not calculated.

Thus from the results of series (a) it can be seen that the root tip has a stronger directional influence on response to gravity than has the root stump, since, with opposite stimulation of tip and stump the direction of curvature is usually determined by the tip. The results of series (a) are seen to be significant since the percentage response obtained is of the same order as that in the control series (b), (c) and (d).

Opposite stimulation of tip and growing region in roots was obtained by Piccard(8) as early as 1904 by means of an apparatus in which centrifugal force was made to act in different directions on tip and stump. The roots were rotated about a horizontal axis in such a manner that a point between the tip and the growing zone was centred above the axis of rotation. Piccard obtained results which showed that both tip and growing zone of the root were sensitive but that the latter was actually more sensitive than the former. Haberlandt(4), using an improved form of Piccard's apparatus, obtained exactly opposite results, the tip being shown to be more sensitive than the growing zone. These experiments are open to considerable objections since the high speed at which rotation must have taken place would introduce many complications such as vibrations, etc. Moreover, the fact that Piccard and Haberlandt obtained such opposite results makes it desirable for the opposite stimulation of tip and stump to be repeated by a method less open to objection. The decapitation

method described above establishes the fact that the root tip is normally more sensitive than the growing region.

A further interesting point, arising from this decapitation experiment, is that, when slightly modified, the experiment throws fresh light on the meaning of geotropic presentation time. Experiments were carried out in which roots of *Vicia Faba* were stimulated *before* decapitation and their tips were then cut off and replaced so that the side of the tip which had been lowest during stimulation was opposite the side of the stump which had been lowest. The roots were then placed in a vertical position. The geotropic presentation time for roots of this strain of bean, of between 6 and 12 cm. long, at a temperature of between 10 and 15° C., was found by experiment to be approximately 1 hour. It was found that if the roots were stimulated for between 1 and 1½ hours and the tips were then reversed as described above, curvature was usually towards the side of the *tip* that was lowest during stimulation. On the other hand, if the stimulation period was more than 1½ hours, curvature was usually towards the side of the *stump* that had been lowest, indicating that some conduction of the stimulation from tip to stump had taken place *before* decapitation. These results suggest that presentation time is the time necessary for the perception of the stimulus by the tip, and that conduction of the stimulus backwards from the tip does not take place until later, i.e. presentation time is the time necessary for the redistribution of sufficient growth hormones in the tip to cause curvature of the growing region.

SUMMARY OF RESULTS

1. It is shown that a greater positive curvature can be induced in an unstimulated root of *Vicia Faba* by hormones extracted from the *lower* halves of a geotropically stimulated root tip than by hormones extracted from the *upper* halves of the same root tips, indicating that growth hormones accumulate in the lower half of the tip of a geotropically stimulated root.

2. It is shown that with opposite gravitational stimulation of growing region and tip in roots, the tip has a stronger directional influence on curvature than has the growing region.

3. Geotropic presentation time appears to correspond to time taken by redistribution rather than conduction of hormones.

My thanks are due to Dr T. A. Bennet-Clark for suggesting these experiments and for giving me the benefit of his advice during their progress.

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FRITSCHIELLA, A NEW TERRESTRIAL MEMBER OF THE CHAETOPHORACEAE

By M. O. P. IYENGAR

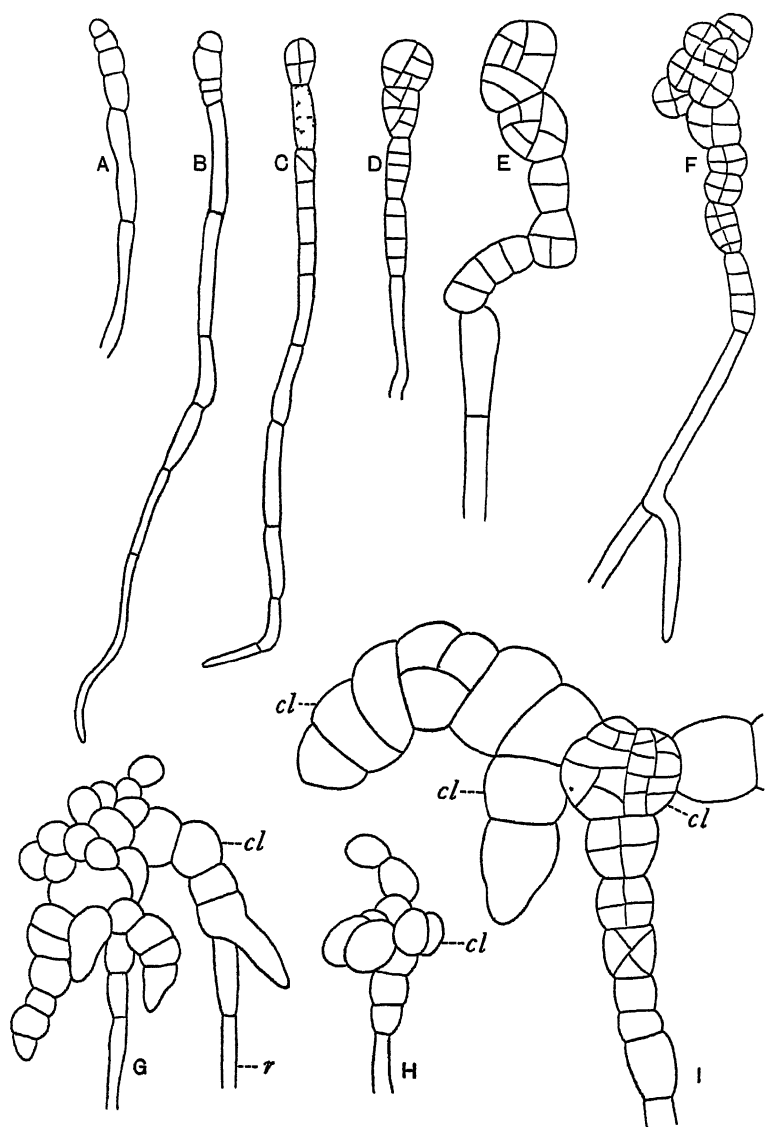
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(With Plate XIII and 2 figures in the text)

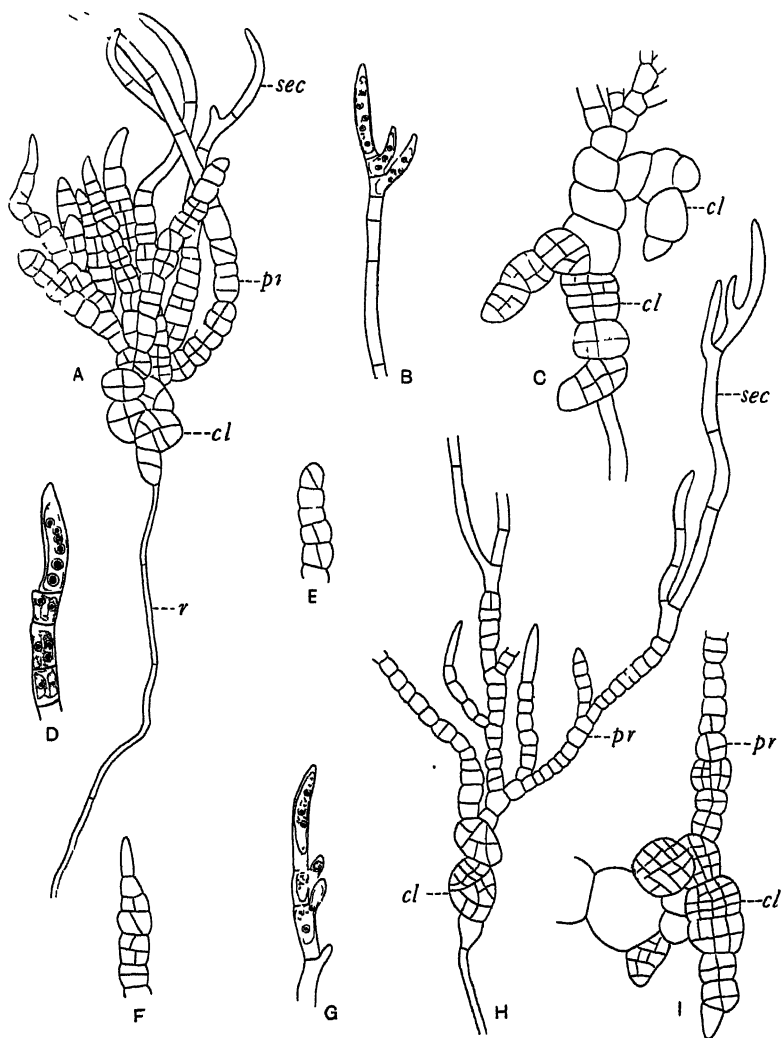
THIS alga was growing more or less gregariously, together with *Protosiphon botryoides* or *Botrydium tuberosum*, on the moist silt of drying rain-water pools at Madras and occurred in a similar situation, together with *Botrydium tuberosum*, at Talguppa in the Mysore Province. The alga has been repeatedly observed in Madras in different years, when the pools were drying up after the north-east monsoon.

At first sight the alga recalls a dense growth of *Stigeoclonium*, but careful examination shows that it possesses a complicated structure which is much more specialised than that of the latter genus. The mature plant consists of (1) a *rhizoidal system*, penetrating the substratum and comprising one or more downwardly directed rhizoid-like filaments made up of much elongated colourless cells with very scanty contents and sometimes with a few branches (Text-fig. 2, *A*, Text-fig. 1, *F*); (2) a *prostrate system* composed of a number of rounded or irregular swollen clusters of cells with dense contents and thin walls, the whole forming an irregular system with short congested branches (Text-fig. 2, *A*, *C*, *H*, *cl*); (3) a *primary projecting system*, arising from the prostrate system and consisting of a number of upright short-celled branched threads (Text-fig. 2, *A*, *H*, *pr*); and (4) a *secondary projecting system* composed of somewhat elongate branches having longer cells with bright green contents (Text-fig. 2, *A*, *H*, *sec*). In the natural habitat only the secondary projecting system arises as a tuft above the surface of the substratum, the remaining portions of the alga being situated on a level with or slightly beneath the surface of the soil.

The development of the mature structure. As far as can be made out from preserved material, the alga seems to develop in the following manner. In the youngest stage it appears as an unbranched erect



Text-fig. 1. *Fritschiella tuberosa* sp.n. A-F, various stages of growth of the young plants. G-I, plants with only the rhizoidal and prostrate systems of clustered cells developed; in G a side branch is forming a second rhizoid. *cl*, clusters of cells belonging to the prostrate system; *r*, rhizoid. E $\times 600$; I $\times 800$; the rest $\times 400$.



Text-fig. 2. *Frittschiella tuberosa* sp.n. A, a small mature plant with a single rhizoid. C, portion of a mature plant. B, D, G, parts of branches belonging to the secondary projecting system. E, F, portions of the primary projecting system. H, part of a plant showing all the various systems. I, a portion of a prostrate system with a branch of the primary projecting system. cl, cluster of cells of the prostrate system; r, rhizoid; pr, primary projecting branches; sec, secondary projecting branches. D $\times 500$; the rest $\times 350$.

filament composed of a few cells and probably largely buried in the substratum. The uppermost cells are somewhat wider with richer contents and form a linear row of four cells (Text-fig. 1, *A, B*). The lower cells are elongate and form a rhizoid which, as mentioned above, may branch once or twice or remain unbranched. Earlier stages than this were not observed.

Each of the four cells terminating this primary filament enlarges and divides into four; the division of the lower cells is generally transverse, while in the upper ones transverse, longitudinal and diagonal divisions are seen (Text-fig. 1, *C-E*). The resulting cells continue to divide in all directions, though mainly along planes at right angles to one another, so that ultimately an irregular group of rounded cell clusters is formed, each cluster being the result of the continuous division of one of the four original cells or of one of their products (Text-fig. 1, *D-F*). In many of these clusters localised growth occurs, so that new clusters are budded out laterally or the whole grows out to one side, usually in a slightly downward direction (Text-fig. 1, *G-I*, Text-fig. 2, *C, I*; Pl. XIII, 1, 3, 5-9). In the latter case the end cell grows out into a long rhizoid (Text-fig. 1, *G*; Pl. XIII, 1, 5). Sometimes a number of rhizoids are formed in this way. In many cases, however, downward growth of the clusters does not occur and then the entire plant has only the single original rhizoid (Text-fig. 2, *A, C, H*; Pl. XIII, 3, 6, 9).

From some of these rounded clusters of cells, representing the *prostrate system* of the alga, but usually from the uppermost ones, a number of filaments grow upwards. These, forming the *primary projecting system*, are composed of short cells whose length and breadth are very nearly equal (Text-fig. 2, *A, H, I[pr]*, *E, F*; Pl. XIII, 1-7), and which have fairly dense contents. Division in these threads mostly takes place transversely, though occasional longitudinal, cross-wise, or diagonal division may occur (Text-fig. 2, *E, F*). Rounded clusters, like those seen in the prostrate system, are, however, never formed. The primary branches show considerable ramification, all the threads growing upwards.

From the upper ends of these primary branches then arise a number of secondary branches (*secondary projecting system*), composed below of much elongated cells with scanty contents and above of comparatively shorter cells with dense bright green contents. The latter, with the exception of the terminal cell, generally grow out laterally into a short branch usually not separated by a septum from the parent cell, though occasionally a septum may be formed

(Text-fig. 2, A, H [sec], B, D, G; Pl. XIII, 2); these branches have a broad conical apex. No hairs of any kind are formed on any of the branches.

In the cells of the secondary projecting system a curved plate-shaped chloroplast with 2-8 pyrenoids is well seen (Text-fig. 2, B, D, G). But in those of the primary projecting and the prostrate systems the contents are very dense and the chloroplast is not clearly recognisable. The pyrenoids in these latter cells are smaller and fewer in number (usually 2-4) than in those of the secondary projecting system. All the cells have a single nucleus. The walls are in no way thickened.

In the material it was not unusual to find plants lacking the secondary system, or possessing only the prostrate and rhizoidal systems (Pl. XIII, 5); such plants represent immature stages which have yet to complete their full growth. Very often one or other of the four systems is especially prominently differentiated. The prostrate system varies very much in the extent of its development. In young plants it is generally small, but it gradually increases in size as the plant grows older, well-grown specimens invariably showing a very well-developed prostrate system.

The rounded clusters of cells constituting the prostrate system and composed of small cells with thin walls and dense contents are very peculiar and hitherto quite unknown among Chaetophoraceae. Their exact function is not clearly established. They remind one to some extent of the swollen upper portions of a *Schizomeris* filament before the walls break down and the contents escape as zoospores. It is probable that they serve for the perennation of the alga during the long dry season.

Algae like *Botrydium*, *Protosiphon* and *Fritschiella* growing on the moist earth of drying pools are always in danger of rapid desiccation, and must be able to meet this contingency in some fashion. As long as the soil is moist they grow and increase in size. When conditions of desiccation set in, the contents of *Botrydium* and *Protosiphon* recede into the underground portions of these plants and form cysts which rest unharmed until the next rainy season. The new plants arise from the contents of the cysts either directly or indirectly by the formation of motile spores. In the case of *Botrydium* and *Protosiphon* the protoplasmic contents can readily migrate into the underground portions as a result of the coenocytic structure. But in the case of *Fritschiella* the septate structure of the alga renders such a migration impossible. It is probable that the cell clusters of the prostrate

system serve as a means of perennation which is prepared *long before it becomes actually necessary* to meet the danger of sudden desiccation. In fact, as already mentioned above, the alga continues to form these perennating clusters of cells from the very beginning of its life, so that they may be available for an emergency at any stage of its existence. The cells themselves with their dense contents and scanty vacuoles appear well fitted for a resting period without further preparation. No special thickening of the walls has been observed, though the outer wall of each cluster was decidedly thicker than the walls between adjacent cells of the cluster. The thin walls of the individual cells of each cluster will facilitate the escape of motile spores, if such, as seems probable, should be formed in these cells when the rainy season commences.

The clusters of cells show a certain remote resemblance to the appearance obtained during the early stages of palmella formation in certain species of *Stigeoclonium*. But in a terrestrial alga like *Frittschiella* the growth of cells, isolated during palmella formation, will lead to a dense growth of new plants at one place. The rather uniformly distributed, though gregarious, growth of the alga on the bed of the pool and the absence of any dense clusters suggest zoospore formation rather than development from a palmella stage.

Numerous plants were carefully examined for empty cells from which motile spores could have escaped, but no such cells were found either in the prostrate or in the projecting systems. It can hardly be doubted that when the soil dries up the projecting system perishes, so that this system is probably purely assimilatory in function, affording an interesting instance of division of labour among the Chaetophoraceae.

In the absence of knowledge of the motile stages of the alga, it is not easy to decide its systematic position. The general appearance of the uppermost branches (secondary projecting system) and of their cells and to some extent also that of the primary projecting system much resemble *Stigeoclonium*. In the possession of rhizoid-like filaments and the terrestrial habit there is similarity to *Iwanoffia* Pascher¹ (*Stigeoclonium terrestre* Iwanoff). It differs markedly from *Iwanoffia*, *Stigeoclonium* and all the other members of the Chaetophoraceae in its special habit, in the formation of a prostrate system composed of clusters of cells that probably perennate and in the high specialisation of its thallus into rhizoidal, prostrate, primary and secondary projecting systems. The alga may therefore be regarded as

¹ A. Pascher, *Bibl. Bot.* 67, 63. 1907.



the type of a new genus of Chaetophoraceae allied to *Iwanoffia* and *Stigeoclonium* which I shall call:

Frittschiella gen.nov.

Thallus terrestrial, branched above and attached to the soil by means of colourless septate rhizoids, with a number of irregularly rounded clusters of small cells with dense contents and thin walls forming a crowded prostrate system probably serving for purposes of perennation, and a projecting system composed of a lower portion consisting of very short cells and an upper portion consisting of elongate cells, bearing some branches; setae absent; zoospores and gametes not observed.

Frittschiella tuberosa sp.n. (Text-figs. 1 & 2; Pl. XIII).

Characters same as for the genus; cells of the upper branches 6–10 μ broad and 3–8 times as long as broad, those of the lower branches 7–11 μ broad and 5–10 μ long; perennating clusters of the prostrate system about 18–40 μ in diameter; rhizoidal filaments 4–8 μ broad; plants without the rhizoids about 250–600 μ high.

Habitat. On moist silt on the bed of drying rain-water pools at Madras, and Talguppa in Mysore Province, India.

In conclusion the writer wishes to express his indebtedness to Prof. F. E. Fritsch, F.R.S. for his guidance and help in preparing this paper.

EXPLANATION OF PLATE XIII

Frittschiella tuberosa sp.n.

1, 3, 4, 6, plants showing habit; 2, primary and secondary projecting systems; 5, young plant with only prostrate and rhizoidal systems developed, with a branch of the primary projecting system beginning to form; 7, 8, a portion of 4 enlarged; 9, a portion of 6 enlarged. *cl* and *d*, prostrate system; *pr*, primary projecting system; *sec*, secondary projecting system; *r*, rhizoid. 7, 9 \times about 200; 8 \times about 350; the rest \times about 100.

GROWTH-REGULATORS IN PLANTS

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(With 5 figures in the text)

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I. THE GROWTH-REGULATOR OF THE COLEOPTILE

THE best known of the growth-regulating substances that occur normally in plants is at present the substance formed by the tip of the coleoptile in seedlings of the grass family, which accelerates the growth of the elongating region of the coleoptile below. The first indication that the tip of the coleoptile normally produces a growth-accelerating substance was obtained by Páal (1919) when investigating the transmission of a phototropic stimulus across a discontinuity—a phenomenon first demonstrated by Boysen-Jensen (1910). It occurred to him, after cutting off the tips of coleoptiles of *Coix lacrima*, to replace them excentrically on the stumps, in the manner shown in Fig. 1. The result was that the coleoptiles curved strongly away from the side covered by the tip, and this he interpreted as indicating that the tip produced a growth-accelerating substance which passed across the moist discontinuity and down the side of the coleoptile that was covered by it, with the result that this side grew faster than the other and caused the coleoptile to curve. Subsequently Söding (1923, 1925, 1929) showed by direct measurement that the growth of decapitated oat coleoptiles

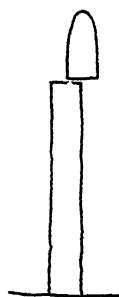


Fig. 1.

was accelerated (within the first 5 hours) by replacing the tips, and he also concluded that the tips form an accelerating substance. But completely definite evidence of this substance was first produced by Went, F. W. (1926, 1928), working in his father's laboratory at Utrecht. Although his main work (1928) is well known and readily accessible, it will be necessary to give here a brief account of some parts of it.

Went's fundamental experiment was to cut off the tips of several oat coleoptiles, and stand them (usually for 1 hour) on a block of agar gel of known size. The block was then cut up into pieces of known size, and these were then placed so that they covered one side of the cut surfaces of the stumps of another set of coleoptiles decapitated just previously. The agar blocks were stuck in position with drops of gelatine. The coleoptile stumps were then observed after 110 or 120 minutes at 25° C., and were regularly found to have curved away from the side covered by the agar block, though control stumps, on which were placed agar blocks on which no tips had been standing, did not curve. This result therefore left no doubt that a growth-accelerating substance or group of substances (which will here be called growth-regulator or G.R.) diffuses out from the coleoptile tip. Further, since very thorough precautions were taken to ensure constancy of temperature, humidity, physiological condition of the seedlings, etc., it was found possible to obtain quantitative results with regard to the rate of formation of G.R., and the effects of different amounts of it, and it is this which gives special value to the investigation. The different amounts of G.R. were supplied by varying the number of tips that stood on each block, or by placing one block with G.R. upon another without any, until the G.R. distributed itself equally between both blocks. In these ways Went was able to show that (below a certain limit) the angle of curvature of the coleoptile stumps varied directly with the amount of G.R. supplied in the agar blocks. This result shows, as will be clear on consideration, that the rate of growth varies directly with the amount of G.R. present, and it was therefore to be expected that if there were no G.R. present, there would be no growth at all. That this is very nearly true was indeed demonstrated by Dolk (as quoted by Went, 1928, p. 65), but the demonstration is less simple than might be expected. For when a coleoptile is decapitated, a considerable amount of G.R. remains in the stump and is used up only gradually, so that the stump continues to grow, though less rapidly than an intact coleoptile. Further, it is of no use to wait for the G.R. to be used up, for after about 3 hours the

uppermost part of the stump begins to act physiologically like a tip. This is the striking phenomenon known as the "physiological regeneration of the tip," which was discovered by Rothert (1894), and confirmed by Söding (1923, 1925, 1929), who showed that after about this time the uppermost part of the stump begins to produce fresh G.R. In order therefore to avoid this complication, Dolk decapitated his coleoptile stumps a second time, after 2 hours. The rate of growth of the stumps sank almost (not quite) to nil, but could be increased again by supplying G.R.

Incidentally it may be noted that the physiological regeneration of a new tip is much retarded by replacing the original tip (Söding, *loc. cit.*) Went's work has formed the starting-point for investigating a number of different questions, which will be considered in the following sections.

2. THE MODE OF ACTION OF THE G.R. IN THE COLEOPTILE

The G.R. must accelerate growth by promoting cell extension and not cell division, for in the elongating zone of the coleoptile the cells have ceased to divide. Went (1928, p. 88) suggested that the G.R. makes the cell walls more plastic, so that they are irreversibly stretched by the turgor pressure of the cell sap beyond their limit of elasticity, and the length of the cell is permanently increased. This suggestion has since received strong support, for Heyn (1931, p. 209 *sq.*) at Utrecht and Söding (1931, 1932 *a*) have shown that if coleoptiles are stretched or bent by small weights beyond their limit of elasticity, the irreversible part of the stretching or bending is much greater in intact coleoptiles than in coleoptiles decapitated about 2 hours previously. Further, Heyn (*loc. cit.*) and Heyn and Overbeek (1931) have shown that if agar containing G.R. is placed upon the decapitated coleoptiles, they behave like intact coleoptiles in this respect. In Söding's experiments indeed the greater plasticity of the intact coleoptiles might have been the *result* of their more rapid growth rather than the cause of it; but this explanation would hardly apply to Heyn's experiments, since these were performed on detached coleoptiles, cut off at the base, which were hardly growing at all (1931, p. 213). In nature, the turgor pressure of the cell sap is fully enough to stretch the walls beyond their limit of elasticity, as is shown very clearly by Söding (1932 *a*, p. 121). The same conclusion was reached previously by Heyn (1931, p. 218), Overbeek (1926, for roots) and Pringsheim (1931). However, it would be premature to conclude that *all* cell extension in plants takes place in this manner.

Indeed, the fact that parts of plants which have been enclosed in gypsum for several days are found to be quite turgorless when first set free (though they recover later), strongly suggests an active growth of the cell-wall, in some tissues at least, as was pointed out by Pfeffer (1893) long ago. Another difficulty is pointed out by Rawitscher (1932, p. 355). The reversible or elastic component of the stretching of the cell wall is not affected by G.R. and appears to be much less important for growth (Heyn, 1931).

3. THE ACTION OF G.R. UPON THE ROOT, AND ITS CONNEXION WITH THE TROPISMS

Cholodny (1924, 1926, 1928), at Kieff, found that coleoptile tips of maize *retard* the growth of decapitated maize roots when placed upon them, and Keeble, Nelson and Snow (1931) confirmed this result. Nielsen also (1930) has found that a certain fungus excretes into its culture-medium a substance which is probably very similar to the G.R. of the coleoptile tip, and this substance also accelerates the growth of coleoptiles, but retards or even arrests that of roots. Thus the elongating zones of coleoptile and root are affected in opposite senses by the G.R. and other similar substances. Root tips also retard the growth of decapitated roots when placed upon them (Cholodny, 1926, p. 458, and 1929, p. 473; Keeble, Nelson and Snow, 1931), and according to Cholodny (1928, p. 126, and 1929, p. 471) they also accelerate the growth of coleoptiles. It therefore appears that the root tip excretes a substance that acts in the same way as that of the coleoptile tip. This conclusion has been definitely established by Hawker (1932) who placed root tips of *Vicia Faba* on blocks of gelatine, and then after some time placed pieces of these blocks excentrically on the cut surfaces of decapitated roots. The roots curved towards the sides covered by the blocks, which showed that the blocks contained a growth-retarding substance derived from the tips, since blocks of pure gelatine caused no curvatures. Keeble, Nelson and Snow (unpublished) have fully confirmed this result, working with maize roots.

These results support the theory of Cholodny (1924, 1926) that the opposite curvatures of root and coleoptile in response to gravity are brought about by the G.R.'s formed by the tips of these organs, which normally travel in straight lines back to the elongating zones and arrive there in equal concentrations on all sides. But as a result of the stimulus of gravity these substances are somehow diverted, as he supposes, so that they travel obliquely and accumulate in greater

concentration on the lower sides of the elongating zones. Went (1928) arrived at a similar theory for the phototropism of the coleoptile. This theory has been summarised in a recent paper by Keeble, Nelson and Snow (1931), which may be consulted for evidence and further references. Weber (1931) points out a difficulty which the theory encounters.

4. THE VARIOUS SOURCES OF G.R. AND ITS CHEMICAL NATURE

Nielsen (1930) found, as already stated, that a certain fungus (*Rhizopus suinus*) excretes into its culture medium a substance which accelerates the growth of coleoptiles and strongly retards that of roots. Various bacteria also (Boysen-Jensen 1931 *a*), yeast (Nielsen 1931 *a*) and the fungus *Aspergillus niger* (Boysen-Jensen, 1931 *b*) form substances which accelerate the elongation of coleoptiles. These substances are all very similar, or possibly the same. They are soluble in ether and, with the G.R. of the coleoptile, may provisionally be grouped together under the name "Growth-substance A" (Nielsen, 1932), or better, "Growth-regulator A" (Boysen-Jensen, 1932). *Rhizopus suinus* forms another substance also which strongly promotes the increase in weight of *Aspergillus niger*, but has no effect on coleoptiles (Nielsen, 1931 *b*, 1932). This substance, which is insoluble in ether and quite different from G.R. "A," may be called growth-substance (or growth-regulator) "B" (Nielsen, 1932). *Aspergillus* itself forms no G.R. "B," but only G.R. "A" (Boysen-Jensen, 1932), although G.R. "A" does not promote the increase in weight of that fungus itself, but indeed slightly diminishes it (*loc. cit.* p. 278). It is only on certain culture media that *Aspergillus* forms G.R. "A."

The chemical nature of G.R. "A" has been very thoroughly investigated by Kögl and Haagen-Smit (1931). They obtained it from various sources, including coleoptile tips of Maize, the fungus *Rhizopus*, yeast and urine. In order to compare the strength of different solutions of G.R., they introduce the term "*Avena* unit" or "A.E." (= *Avena*-Einheit). One such unit is defined as being the amount of G.R. which, when contained in a block of 3 per cent. agar measuring $2 \times 2 \times 0.5$ mm., imparts a curvature of 10 degrees in 2 hours, at 22° or 23° C., to one decapitated coleoptile of *Avena* (the oat), upon which the agar block is placed excentrically as in Went's experiments. They found that from the coleoptile tips there diffused out a solution containing 300 A.E. per mg.: urine contained 400 A.E. per mg. From urine they succeeded finally in isolating a purified crystalline substance which had an efficiency of 30,000,000 A.E. per

mg. In other words, 1 milligram of this substance was enough to impart a curvature of 10° to 30,000,000 *Avena* coleoptiles. They consider that the G.R. in urine may be produced by bacteria in the digestive tract. Saliva also strongly accelerates growth (Seubert, 1925; Fliry, 1932). The G.R. appeared to be the same from whatever source it was obtained. Three determinations of its molecular weight came to 342, 353 and 330. These figures agree well with an estimate of roughly 376, made by Went, which was based on its rate of diffusion. The G.R. is soluble in ether and other organic solvents and is not destroyed by heat. It contains no nitrogen, sulphur or phosphorus. The authors conclude that it is an aliphatic carbon compound.

5. THE TRANSPORT OF G.R. IN COLEOPTILES

Went (1928, p. 57) discovered that in the coleoptile the G.R. can scarcely travel at all except in the morphologically downward direction. He cut out cylinders of coleoptile a few millimetres long (from 2.3 to 4.2 mm.) and on the upper ends of these cylinders he placed blocks of agar containing (to use a convenient expression) known "concentrations" of G.R. (The "concentration" of G.R. in a block of agar of given size may be defined in terms of the amount of curvature which the block would produce if placed excentrically on a decapitated coleoptile (see van der Weij, 1932, p. 395¹).) The lower ends of the cylinders rested on blocks of pure agar. After given times, in one experiment 75 minutes, the blocks were removed and placed excentrically on other decapitated coleoptiles, in the usual way, and the curvatures produced were measured. In this way it was determined how much G.R. had reached the lower block during the experiment, how much had left the upper block, and incidentally (by subtraction) how much had been used up in the cylinders. It was found that, when the cylinders were normally orientated, a very considerable part of the G.R. was transported into the lower block (much more than could be accounted for by diffusion), while a certain amount was used up in the cylinder. On the other hand, when the cylinders were inversely orientated, so that the upper blocks, containing the G.R., rested on the morphologically lower surfaces,

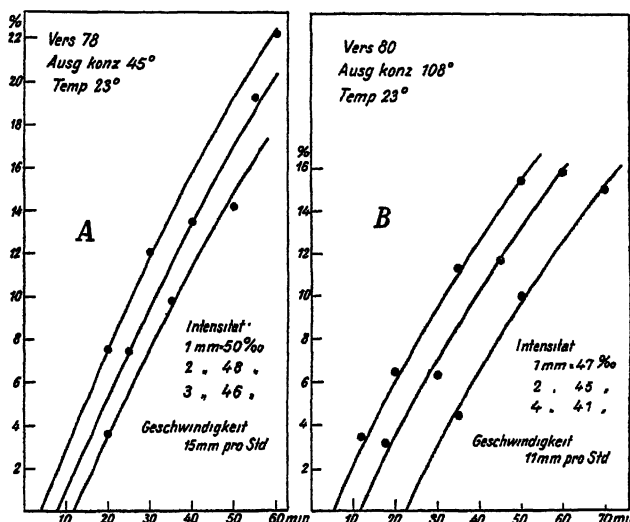
¹ Went himself does not use this expression. Indeed he claimed (1928, pp. 37-8) that the curvatures varied directly with the absolute amount of G.R. in the agar block and not with its concentration. But van der Weij (1932, pp. 393 *sq.*) found on the contrary that the curvatures varied directly with the concentration of G.R. if the blocks were of different sizes. The point makes no difference, however, to most of Went's results, including the present ones, since his blocks were nearly always of the same size.

practically no G.R. was transported to the lower blocks. The transport of G.R. in the coleoptile is therefore polar, taking place only in the morphologically downward direction. Beyer (1928, p. 330) demonstrated this fact independently in a different way. It might indeed be objected that the failure of the inverted cylinders to transport G.R. might be due to an effect of gravity upon them. But this is not so, for van der Weij (1932, p. 445) repeated Went's experiment and also performed similar experiments with the whole system inverted in relation to gravity, so that the blocks containing G.R. were below and the blocks of pure agar above. He found that the direction in which gravity was acting made very little difference, if any, to the result.

The G.R. must therefore be transported in a manner quite different from that in which the commoner organic nutritive substances are transported. For the latter, which, as is known, travel mainly in the phloem, can travel in either direction according to the circumstances. (For discussion of the transport of these substances see Münch, 1930.) Also the path by which the G.R. is transported is different, since its transport does not depend on the two small bundles of xylem and phloem, of which one is present on each of the narrower sides of the coleoptile. For if an agar block containing G.R. is placed excentrically on a decapitated coleoptile above one of the narrower sides, the curvature produced is actually less than if the block is placed on one of the broader sides between the bundles (van der Weij, 1932, p. 488).

Concerning the manner of transport of the G.R. some very interesting further facts have been discovered by van der Weij (1932), also at Utrecht. His method of investigation was based on that of Went: for he cut out cylinders of coleoptile, and placed on top of them blocks of agar containing known concentrations of G.R. The lower end of each cylinder rested on a block of pure agar, and after a given time this block was tested, to determine how much G.R. had reached it, by being placed excentrically on another decapitated coleoptile in the usual way. With this arrangement he tested how the transport of G.R. in the cylinders was affected by varying the following factors: (1) length of cylinder, (2) temperature, (3) initial concentration of G.R., (4) orientation of the cylinder. His usual source of G.R. was a solution of known strength obtained from urine and supplied by Kögl and Haagen-Smit. In order to state his results, it is necessary to distinguish between what he calls the "velocity" and the "intensity" of transport. The "velocity" is measured by the time that

elapses before the first appreciable trace of G.R. reaches the lower block after travelling through a coleoptile cylinder of known length. The "intensity" is measured by the amount of G.R. that subsequently reaches the lower block per unit of time. The necessity for distinguishing between these quantities will be clear if one reflects that for some time after the start of the experiment it will not be possible to detect any G.R. in the lower block, so that during this period no "intensity" of transport can be measured. After this one may expect a transitional period during which the "intensity" of transport will rise to a steady value at which it will remain, except in so far as it may be affected by changes in the concentration of G.R. in



Figs. 2 and 3.

the two blocks. Actually the results showed quite clearly the difference between the first and last of these three periods, though the transitional period was not detected and may have been very short.

In order to determine the effect of varying the length of the path of transport, van der Weij used coleoptile cylinders of various lengths, and determined the amounts of G.R. that reached the lower blocks after times varying from 10 to 100 minutes. The results of two experiments are shown graphically in Figs. 2 and 3. The three curves in each graph are drawn through points representing the amounts of G.R. (measured in terms of the degrees of curvature that they cause) that were transported to the lower blocks through cylinders of three

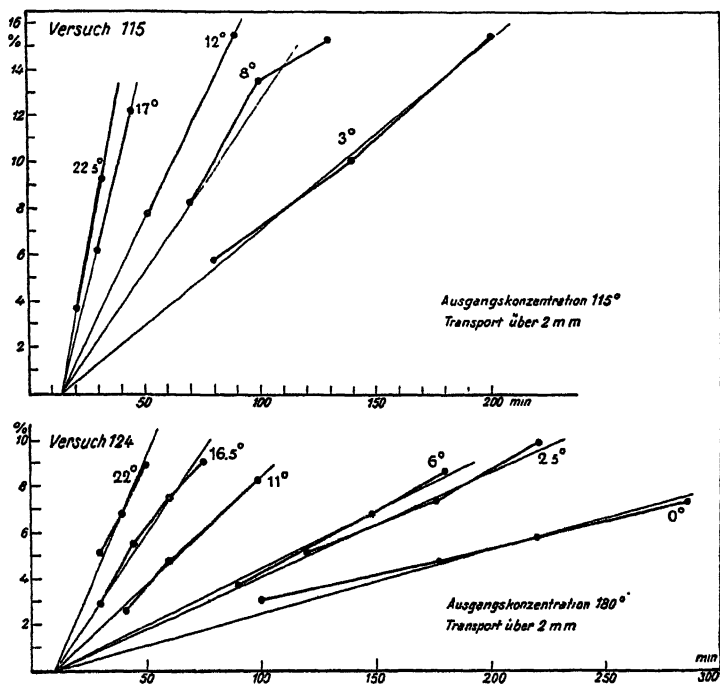
different lengths after various times. The lengths of the cylinders were 1, 2 and 3 mm. in one experiment, and 1, 2 and 4 mm. in the other. Ordinates show amounts of G.R., abscissae show time. It can be seen that the curves are very nearly parallel, which shows that *the "intensities" of transport through paths of different length are almost equal*. This striking result shows clearly the contrast between the transport of G.R. and simple diffusion, since in diffusion the intensity varies inversely with the length of path. Other experiments gave similar results. If the curves are continued downwards, they cut the abscissae at points whose distances from the origin are approximately proportional to the lengths of the paths of transport. From this van der Weij concludes that the "velocities" of transport also are independent of the length of path. But it should be remembered that this conclusion is based on an extrapolation. The velocities ranged from 10 mm. to 15 mm. per hour in various experiments.

In order to determine the effects of different temperatures, van der Weij performed similar transport experiments at temperatures ranging from 0° C. to 40°. When the results were expressed graphically, as in the last experiment, the curves showing the results at different temperatures were found not to be parallel. The steepest curve (indicating the greatest "intensity" of transport) was obtained at 30° C., and those obtained at temperatures above and below this were progressively less steep. The "intensity" therefore varies with temperature in the same way as many physiological functions, reaching a maximum at about 30° C., and falling off above and below this temperature. There was still distinct transport at 0° C. On the other hand, as regards the "velocity" of transport the conclusion indicated is quite different. For if the curves are continued downwards, they cut the base line at approximately the same point. Graphs of two experiments are shown in Figs. 4 and 5. The extrapolation no doubt again involves some uncertainty, but the impression derived from the graphs of these and other experiments is certainly very strong that if values nearer the base line could have been obtained, they would indeed have been very close together. But if this is so, the surprising conclusion follows that *the "velocity" of transport is nearly independent of temperature*.

The velocity of transport therefore probably does not depend on the rate of protoplasmic streaming (van der Weij, p. 478), since the latter has a high temperature coefficient¹. Also, since the cyto-

¹ This argument would not hold if the time taken in moving with the streaming protoplasm was very small in comparison with the time taken in crossing the cell walls.

plasm of the coleoptile cells does actually stream fairly rapidly (Went, 1928, p. 56), it further seems probable that the G.R. is transported mainly in an outer stationary layer of cytoplasm in each cell or in the young cell wall. This seems indeed quite plausible, since it is on the young cell wall that the G.R. produces its effects, though probably only in co-operation with the cytoplasm. But the mechanism of transport remains very difficult to understand: a simile given by van der Weij (p. 482) helps one at least to fix the facts in mind. Imagine a service of lorries which continually carry sand (representing



Figs. 4 and 5.

the G.R.) from one place to another, and suppose then that these lorries travel at the same pace whatever the temperature, but that the amount of sand carried by each one varies with the temperature. But how can these varying "loads" be understood? van der Weij suggests (p. 490) that the micellae of the cell wall move further apart at favourable temperatures, and so provide a broader path for transport. If this is so, a better simile would be to suppose that at favourable temperatures several lorries travel abreast, but at less favourable temperatures fewer can travel abreast.

As to the effect of the initial concentration of G.R., van der Weij was able to show that the "intensity" of transport increases with increasing initial concentration, but considerably less rapidly. On the other hand the "velocity" of transport appears to remain unaltered. Finally he succeeded in showing the polarity of transport in a new and striking way. Since the G.R. was transported only in one direction, it occurred to him to ask what would happen if the experiments were continued for longer periods. He found that the concentrations of G.R. in the lower blocks of agar continued to rise until they were much higher than in the upper blocks in which the G.R. was contained at the start. Thus in one experiment the upper blocks were given initial concentrations of G.R. of 10° (see page 341), while the lower blocks contained no G.R. After $4\frac{1}{2}$ hours, the mean concentration in the upper blocks was found to be only 2.7° , and that in the lower blocks 7.4° . In another experiment, the upper blocks were again given 10° , but the lower blocks were given 30° : after $4\frac{1}{2}$ hours the upper blocks only contained 2.8° . By further experiments like the last, van der Weij was able to show that the transport away from the upper blocks was not appreciably retarded even when the lower blocks contained at the start sixteen times as much G.R. as the upper. (The lower blocks were not tested at the end of these experiments on account of the difficulty of detecting the arrival of comparatively small amounts of G.R. in blocks which contained such high concentrations at the start.) Above this limit, transport from the upper blocks began to be retarded, but even this may have been an error due to diffusion in films of water on the surfaces of the cylinders, which were only 2 mm. long. The author refers briefly, for comparison, to some of the well-known instances in animal physiology in which substances are secreted against a concentration gradient. For many other interesting points and for the elaborate technical arrangements, the original paper must be studied.

6. THE ACTION OF G.R. ON SHOOTS

Under this heading unfortunately not much is yet known. Beyer (1925, p. 695) performed experiments on sunflower seedlings which indicated that the elongation of the hypocotyl is accelerated by substances coming from the shoot tip (not from the cotyledons). Fliry (1932) has thoroughly confirmed this conclusion in the following way. The hypocotyls of sunflower seedlings are strongly and permanently retarded in their elongation by decapitation. But if the shoot tip is stuck on again with gelatine, then the rate of elongation

can be increased again even up to the normal. Also if the tips are allowed to stand on blocks of agar for 2 or 3 hours, and the blocks are then placed on the cut surfaces of decapitated hypocotyls, the rate of elongation can be restored to the normal, though pure agar has no effect. The growth-promoting substance must come from the shoot tip, and not from the cotyledons, since removing or replacing the cotyledons has very little effect. Söding (1926) showed that the elongation of various inflorescence stalks was retarded by decapitation, but could be accelerated again by replacing the young inflorescence on the cut surface in moist contact. Uyldert (1927), at Utrecht, confirmed this result for the common daisy (*Bellis perennis*), and further showed that agar containing G.R. obtained from oat coleoptiles accelerated the elongation of the daisy stalks quite as well, which suggests that all these elongation-promoting substances are at least closely related.

In species of *Tradescantia*, which have a zone of intercalary growth at the base of each internode, the elongation of an internode is greatly retarded by decapitation. Its power of responding to geotropic stimulation is also much diminished by decapitation or by removing the leaves (Uyldert, 1931, p. 40). The rate of elongation and the geotropic response of these decapitated internodes can be greatly increased by placing on the cut surfaces tubes containing agar with G.R. derived from maize coleoptiles (Uyldert, 1931). Also the geotropic response of defoliated stems of *Bryophyllum* can be increased by placing agar with G.R. on the cut surfaces of the petioles (Uyldert, 1931, p. 45). It therefore seems probable that the leaves of these plants promote the elongation and geotropic response of the internodes below them, by forming G.R., or some related substance, which travels from them down the stem. However, Uyldert was not able to extract any G.R. from *Tradescantia*. The fact that, in *Bryophyllum*, the leaves promote the geotropic response of internodes below them was demonstrated previously by Loeb (1924, p. 74). In *Tradescantia* the node, even without its leaf, does the same, though less strongly (Uyldert, 1931, p. 45).

It seems to be a fairly general rule (except in climbing plants) that leaves, and especially growing leaves, promote the elongation of neighbouring internodes. Tammes (1903) showed this clearly for many woody shoots, and showed further that the influence of each leaf usually extended to one or two internodes above it as well as below it. The fundamental question, therefore, is whether this influence of the leaves is regularly due to their producing either G.R. or

some related substance. If so, the further question will arise whether in shoots the G.R. can travel upwards as well as downwards, since in many shoots the leaves accelerate the internodes above them also. Just possibly in these shoots the G.R. may move upwards in the vessels of the wood. A wide field here lies open for investigation. In *Tradescantia* indeed the influence of the leaves seems to travel mainly or entirely downwards, but the point needs to be further investigated. Also in sunflower hypocotyls the experiments of Beyer (1925, pp. 695-6) indicate that the G.R. travels downwards only.

7. ROOT-FORMING AND OTHER SUBSTANCES

Went, F. W. (1929), working in Java, made the valuable discovery that certain leaves form a soluble substance which promotes the formation of roots by stem cuttings. He thus provided support for a well-known suggestion made long ago by Sachs (1882, p. 694) that the general tendency of cuttings of stem or root to form roots at the base is due to a root-forming substance which tends to travel downwards in the stem. Went's experiments were briefly as follows. Several leaves of *Acalypha* or *Carica Papaya* were placed with their stalks in a small quantity of water. The water was boiled down every day at low temperature, and replaced by fresh water. After some days the water, with the substances exuded into it, was mixed with warm 3 per cent. agar, which was allowed to solidify and was applied to the sides of leafless woody stem cuttings of *Acalypha*, near the top, after removal of the cork. These cuttings were found to form roots at the base more quickly than untreated controls: (for further details see the original). Diastase also promoted root formation in a similar manner: it even did so after it had been boiled, so that its action must have been due to some accessory substance, and not to the enzyme itself. Went claims (1932, p. 529), from unpublished results, that the root-forming substance moves downwards only.

These results naturally raise the question, what is to be thought of the other part of Sachs' suggestion—that the tendency for the growth or formation of buds to be strongest at the upper end of a cutting is due to a substance for bud growth that tends to move upwards? It is not an insuperable objection that in stems the G.R. extracted from coleoptiles travels downwards at least as easily as upwards—indeed probably more easily. For a substance promoting bud growth might well travel differently, since it might have quite different properties, and act by promoting cell division and leaf

development in buds, whereas the G.R. of the coleoptile influences only cell extension. Here again are opportunities for experiment.

Incidentally it may be noted that Nemeč (1930) claims to have found evidence of a substance specifically *preventing* bud formation. For he found that if fresh cultures of *Bacterium tumefaciens* were applied to the upper cut surfaces of root cuttings of *Cichorium intybus*, they quite prevented the usual formation of buds at that place, and promoted instead a strongly growing callus from which even roots were formed after some time. But it seems not altogether impossible that these may have been the effects (direct and indirect) of the mitogenetic radiation which this bacterium is known to emit (Magrou, J. and M., quoted by Gürwitsch, 1932, p. 72). The experiments might be repeated with dead cultures.

8. THE CAMBIAL STIMULUS AND THE POLAR TRANSPORT OF HORMONES

The leaves, and especially the growing leaves, of dicotyledons strongly promote cambial growth in the stem below them. They do so even in seedlings that have been grown continuously in the dark from the start, so that it cannot be through the products of their photosynthesis that they do so. Their influence on cambial growth, which it will be convenient to call "the cambial stimulus," is transmitted in the morphologically downward direction only, and cannot be explained by their pull on the transpiration stream. These important facts were demonstrated by Jost (1891, and especially 1893). There are indeed some observations which go to show that in the trunks of some broad-leaved trees cambial growth starts in spring before the leaves begin to grow (see Büsgen-Münch, 1927, p. 99). Priestley (1930, pp. 323, 324), on the other hand, maintains that such observations have been wrongly interpreted, and that in the above-ground parts of broad-leaved trees, cambial growth always spreads downwards from growing buds or leaves. But even if in some trees cambial growth starts before leaf growth, this does not diminish the interest of the facts stated above concerning the cambial stimulus: for there is little doubt that in these trees, as in other plants, the growing buds and leaves subsequently accelerate the growth of the cambium below them.

It was suggested by Kastens (1924) that the cambial stimulus is a hormone, and the fact that it travels only downwards, like the G.R. in the coleoptile, supports this suggestion. Unpublished experiments

by the writer have recently shown that it can pass across a protoplasmic discontinuity and even through an interposed piece of moist linen, so that the probability that it is a hormone is very strong: for the rate at which it travels is much too slow for it to be propagated by relays of secondary mitogenetic radiation in the manner discovered by Gürwitsch (1932, pp. 279 *sq.*). As to the rate at which the cambial stimulus travels, the evidence is indeed very confused. But several observations indicate that in forest trees it takes some weeks to travel down from the growing leaves near the top to the base of the trunk. Thus according to Hartig (cited by Büsgen-Münch, p. 101) in larches and maples in spring the cambium starts growing near the tips of the twigs four weeks earlier than in the lower part of the trunk. If therefore one assumes a distance of 20 metres from the buds to the base of the trunk in these trees, this gives a velocity of approximately 3 cm. per hour for the transmission of the cambial stimulus, which is not greatly different from the velocity of 1 to 1.5 cm. per hour found by van der Weij for the transport of G.R. in coleoptiles. For other observations see Büsgen-Münch, pp. 101, 102.

As to the path of the cambial stimulus, simple experiments on regeneration in herbaceous plants (for instance *Vicia Faba*) indicate that it can travel through unspecialised parenchyma, so that very probably it can travel through most living tissues. In trees it may travel largely in the cambium itself, as suggested by Söding (1932 *b*). It cannot be with the sap of the sieve tubes that it travels, for this can move in either direction (Münch, 1930, p. 128). Two further incidental points may be noted. Firstly, the cambial stimulus is quite different from the G.R. of the coleoptile, since the former promotes cell division and the latter cell extension. Secondly, cambial growth takes place not only below growing leaves, but also below growing inflorescences and fruits. For in the axes of leafless inflorescences of, for instance, *Agrimonia eupatoria*, *Spiraea ulmaria* and *Scrophularia nodosa*, it can readily be observed that the amount of secondary wood increases greatly towards the base. Also in *Scrophularia nodosa* lateral fruiting branches, with well-developed fruits, show up to three times as much secondary wood as branches nearer to the apex, bearing equal numbers of younger fruits. It seems therefore that growing flowers and fruits stimulate cambial growth, in some plants at least. Priestley (1930, p. 327) states that the flowers of the ash promote cambial growth.

The polar movements of cambial stimulus and G.R. are rather puzzling when considered together. For in the stem the former

travels downwards only, as was shown by Jost (1893) and also by the writer in a different manner (1932, p. 100). It also travels only downwards in roots, as the writer finds in *Vicia Faba*, and in the leaves it must travel downwards since it travels from them into the stem. As far as concerns transplantation also, stem, root and leaf are "polarized" in the same sense. For Vöchting's famous experiments (1892) showed long ago that if a piece from one of these parts is to be transplanted successfully into a different part, it needs to be kept in its normal morphological orientation. On the other hand, the G.R. which in coleoptiles travels downwards only, must travel upwards in roots. For the G.R. coming from root tips or coleoptile tips which are placed upon the cut surfaces of decapitated roots must travel up those roots as far as the growing zones whose elongation it retards. The question whether in roots the G.R. travels upwards actually better than downwards is now being investigated by Keeble, Nelson and Snow. Thus whatever forces are invoked to explain these polar movements, it will need to be explained how it is that the cambial stimulus travels in the same direction in stem, leaf and root, whereas the G.R. can travel in opposite directions in root and coleoptile.

Recently Went, F. W. (1932), working in Java, has propounded a theory of polarity according to which the G.R. and the root-forming substance are driven downwards electrolytically in all parts of plants by potential gradients. He considers that the G.R. dissociates as an acid, so that its organic part would be an anion. Since the velocity of the transport of G.R. in coleoptiles is much too great to be explained by electrolytic transport over the whole distance, Went supposes (p. 544) that within the cells the G.R. is moved by protoplasmic streaming, but that it is transported across the walls between the cells by local potential differences. He seeks to get over the difficulty of the upward movement of the G.R. in roots by denying that the root tip forms any G.R., and then trying to explain in the following manner (p. 546) the admitted fact that root tips (or coleoptile tips) retard the growth of decapitated roots, when placed upon them. He supposes that G.R., formed in the parts above ground, travels all the way down to the elongating zone of the root, which it retards. If now the root has been decapitated, he supposes that some of the G.R. escapes by exudation from the cut surface; but if a tip has been replaced on the root stump, he supposes that it "blocks" the cut surface, so that more of the G.R. remains in the stump.

In spite of the writer's admiration for Went's earlier work, it

seems to him that this suggestion is untenable for the following reasons, amongst others.

(1) Hawker (1932), as stated in section (3), using the method of extraction into gelatine, has shown conclusively that the root tip does form a substance which can travel up the root and retard it on one side. Keeble, Nelson and Snow (unpublished) have confirmed this result.

(2) The experiments of Keeble, Nelson and Snow, which showed that a replaced root tip retards the root stump, were performed with the roots in damp air, as were also those of Cholodny. Although, therefore, the cut surfaces were often found to be just moist with exuding water, this water can seldom (if ever) have been so plentiful as actually to drip away off the stumps of the decapitated roots: for if it had done so, then the tips which had been placed on the stumps would have been washed away from their proper positions, which seldom happened. (The tips were only placed in contact, and not stuck on with gelatine.) How then can any G.R. be supposed to have been lost from the stumps? Further, if the exuding water *had* been so plentiful as to drip, the tips would have provided no obstacle, since they would have been washed away.

(3) If blocks of paraffin wax are placed eccentrically on the cut surfaces of decapitated maize roots, they adhere, but the roots do not curve, as found by Keeble, Nelson and Snow (unpublished), in experiments on 40 roots.

(4) Went's suggestion cannot account for the perception of geotropic stimulus by a root tip, nor for its transmission across a discontinuity to an unstimulated stump (see Keeble, Nelson and Snow, 1929).

Even apart from the question of the root, Went's theory and some of his arguments seem to the writer to be open to criticism: but discussion would lead too far. It is not disputed however that electrical forces may be involved in some way or other in the transport of hormones.

It may be mentioned that Söding (1932 *b*) in a very recent short but useful review has discussed some questions not mentioned here. An excellent earlier review by Stark (1927) also contains many other interesting results.

SUMMARY

1. In coleoptiles, the tips form a substance which accelerates cell extension in the elongating zone below.

2. This substance (here named "growth-regulator" or G.R.) increases the plasticity of the cell walls.

3. Root tips also form G.R., or a similar substance. The G.R. *retards* the growth of roots. On the basis of this fact, the opposite tropisms of root and coleoptile can be explained.

4. G.R. is also formed by bacteria and fungi. It is effective in very low concentration.

5. Several unexpected facts are recorded concerning the transport of G.R. in coleoptiles. The manner of transport of hormones in plants is quite different from that of the nutritive substances.

6. Certain shoot-tips and young inflorescences form a substance, probably similar to G.R., which promotes the elongation of the stem below them.

7. Leaves and buds form a substance which promotes the formation of roots in cuttings.

8. Growing leaves promote cell division in the cambium: their influence travels only in the morphologically downward direction. In coleoptiles, the G.R. also travels downwards only, but in roots it travels upwards as easily as downwards, or perhaps more easily.

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